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(71) Applicant: COCENSYS, INC. [US/US]; 213 Technology Drive, Irvine, CA 92718 (US).

(72) Inventors: GEE, Kelvin, W.; 47 Urey Court, Irvine, CA 92715 (US). LAN, Nancy, Tsai-Yun; 522 Hermosa Street, South Pasadena, CA 91030 (US).

(74) Agents: WOLFF, Jessica, R. et al.; Lyon & Lyon, 34th floor, 611 West Sixth Street, Los Angeles, CA 90017 (US).

(54) Title: METHODS AND COMPOSITIONS FOR INDUCING SLEEP

(57) Abstract

Methods and compositions for interacting with the $GABA_A$ receptor complex to induce sleep in humans using certain 5 3α-hydroxy-5-reduced steroid derivatives.

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DESCRIPTION

Methods And Compositions For Inducing Sleep

Reference to Related Applications

This application is a continuation-in-part of copending application Ser. No. 08/068,378 filed May 24, 1993, which is a continuation-in-part of Ser. No. 745,216 filed August 13, 1991, issued as Patent No. 5,232,917 on August 3, 1993; which in turn is a continuation-in-part of application Ser. No. 521,724, filed May 10, 1990, issued as Patent No. 5,120,723 on June 9, 1992; which in turn is a continuation-in-part of application Ser. No. 379,047 filed July 13, 1989, now abandoned; which is a continuation-in-part of Ser. No. 089,362, filed August 25, 1987, now abandoned. This application is also a continuation-in-part of Serial No. 196,919, filed February 14, 1994; which is a continuation-in-part of Ser. No. 846,193 filed March 4, 1992 (allowed) which is a continuation-in-part of Patent No. 5,232,917, issued on August 3, 1993, whose priority is described above. Ser. No. 196,919, filed February 14, 1994 is also a continuation-in-part of Serial No. 101,497 filed August 2, 1993, which is a continuation of Patent No. 5,232,917 whose priority is described above. This application is also a continuation-in-part of Serial No. 196,972, filed February 14, 1994 which is a continuation-in-part of Serial No. 101,497 filed August 2, 1993 whose priority is described above.

Background

The present invention is directed to methods for interacting with the gamma-aminobutyric acid receptor-chloride ionophore complex ("$GABA_A$ receptor complex") to induce sleep in humans. More particularly, the present invention relates to the use of certain 3α-hydroxy-5-

reduced steroids and derivatives thereof in the treatment of insomnia.

The relationship between steroids, the $GABA_A$ receptor complex, and brain excitability has previously been described in U.S. Patents Nos. 5,120,723 and 5,232,917 which are incorporated herein by reference in their entirety. The inventors have found that certain steroids, called neuroactive steroids, acting at the steroid site on this receptor complex can be used to induce sleep and in particular, treat insomnia. For example, the inventors have found that 3α-hydroxy-5β-pregnan-20-one ("pregnanolone"), can induce sleep when administered orally. Arafat and co-workers had established a correlation between the progesterone metabolites pregnanolone and 3α-hydroxy-5α-pregnan-20-one and sleep following administration of progesterone in humans. Arafat, E.S., Hargrove, J.T., Maxson, W.G., Desiderio, D.M., Wentz, A.C., and Anderson, R.N. "Sedative and Hypnotic Effects of Oral Administration of Micronized Progesterone May Be Mediated Through Its Metabolites" Am. J. Obstet. Gynecol. 159:1203-1209 (1988). The oral activity of pregnanolone is surprising since Laszlo Gyermek found that it was almost inactive orally in "Pregnanolone: a Highly Potent, Naturally Occurring Hypnotic-Anesthetic Agent" Proc. Soc. Exp. Biol. Med. 1967, 125:1058-1062.

No incentive existed, however, to make an orally active formulation of pregnanolone because earlier investigations indicated that the neuroactive steroids acted like the barbiturates, which exhibit the undesirable side effect of anesthesia when used to induce sleep, a low therapeutic index, abuse potential, and adverse side effects such as rebound insomnia. Majewska, M.D.; Harrison, N.L.; Schwartz, R.D.; Barker, J.L.; and Paul, S.M. "Steroid Hormone Metabolites Are Barbiturate-Like

Modulators of the GABA Receptor" Science 232:1004-1007, 1986.

It has been known since 1967 that progesterone, an anesthetic steroid, when administered at subanesthetic doses could induce sleep in the cat. Heuser, G. "Induction of Anesthesia, Seizures and Sleep by Steroid Hormones" Anesthesiology 28:173-183 (1967). Heuser concluded that progesterone produced a "natural" sleep in the cat because REM sleep was not suppressed during administration of the drug, nor was REM sleep increased upon withdrawal of the drug. Significantly, Heuser found that not all anesthetic steroids were capable of producing this "natural" sleep.

The National Commission on Sleep Disorders Research estimates that 40 million Americans suffer from chronic sleep disorders such as insomnia, narcolepsy, and sleep apnea. It is estimated that 20-30 million Americans experience intermittent sleep-related problems. "Wake Up America: A National Sleep Alert" Vol. 1, p. vi; Draft Report of the National Commission on Sleep Disorders Research 1992. The large incidence of insomnia and other sleep problems is not surprising when one considers that normal sleep-wake patterns can be interrupted by emotional distress, drugs, jet lag, shift work, illness, and strong sensory stimuli such as pain or hunger.

The social costs of this high incidence of sleep disorders and sleepiness are significant. These costs include reduced productivity, lowered cognitive performance, increased likelihood of accidents, higher morbidity and mortality risk, and decreased quality of life. Id. The Commission estimated that in 1990 alone, sleep disorders and sleepiness cost the United States at least $15.9 billion in direct costs alone, which does not include indirect costs from sleep related tragedies such as the grounding of the Exxon Valdez. Id. Society

desperately needs safe and effective treatments for sleep disorders.

Currently, insomnia is treated with a variety of drugs, all of which have certain drawbacks. A common treatment is the use of the drug Triazolam, known by the tradename Halcion. Its drawbacks include rebound insomnia and limited efficacy in increasing sleep. Rebound insomnia is a return of abnormal sleep patterns as the drug wears off or as a result of either tolerance to the drug or withdrawal symptoms when the treatment is stopped. Zolpidem, a new insomnia drug, also tends to cause rebound insomnia.

The compounds of the present invention have superior characteristics to currently available drugs. The present invention provides an insomnia treatment with rapid onset of sleep, high potency and efficacy, and minimal rebound insomnia.

To aid in fully understanding the present invention, a brief outline of sleep and how it is studied follows. Sleep is often defined using two sets of criteria: 1) electrophysiological; and 2) behavioral. The electrophysiological criteria can include electroencephalograms (EEGs), electro-oculograms (EOGs), and electromyograms (EMGs). The most common criterion used for studying sleep is the EEG which is a recording of the electrical activity of the brain. By studying cortical EEGs of sleeping individuals, scientists have found that there are two major stages of sleep manifested by the production of different electrical wave patterns. These two stages are called non-REM (NREM) and REM. NREM sleep is conventionally divided into four stages in which the EEG pattern is commonly described as synchronous, with characteristic waveforms such as sleep spindles, K complexes and high-voltage slow waves. Kryger, M.H.; Roth, T.; and Dement, W.C. Principles and Practice of Sleep Medicine, p.3, 1989 (hereinafter "PPSM"). REM sleep generally shows greater

EEG activation, muscle atonia, and episodic periods of rapid eye movements. Id.

When an individual closes his or her eyes in preparation for sleep, the cortical electrical rhythms synchronize to a rhythm of 8-13 Hz. This is known as an α-rhythm. An EEG taken in the first stage of sleep shows a slower rhythm (4-6 Hz) and a lower amplitude. Bowman, W.C., and Rand, M.J. Textbook of Pharmacology, p 6.24, 2nd ed. 1980 (hereinafter Bowman and Rand). In the second stage of sleep, more irregular and slower EEG waves of 1-5 Hz with a larger amplitude appear. These irregular, slow waves are interspersed with bursts of faster waves called sleep-spindles. In this second stage of sleep there are also K-complexes which consist of a small sharp wave, followed by one or two larger waves and then by faster waves of about 12 Hz. Id. Stage 2 accounts for 45-55 % of sleep time. PPSM at p. 9.

Stage 3 sleep is characterized by high voltage, slow EEG wave activity accounting for 20-50 % of the EEG activity in this stage. PPSM p. 7. In stage 4, high voltage, slow wave activity accounts for >50% of the EEG, and K-complexes cannot be evoked by stimuli such as calling the subject's name. PPSM at p. 7; Bowman and Rand p. 6.24.

After about an hour of sleep the EEG pattern speeds up to a 4-10 Hz rhythm of low amplitude waves that are desynchronized. This is REM sleep and is often referred to as paradoxical sleep because the EEG resembles that of wakefulness, yet it is very deep sleep. Bowman and Rand p. 6.24. The biological significance of REM sleep is not yet known. However, alcohol's strong inhibition of REM sleep has been associated with sleep disruption. Triazolam and other insomnia drugs reduce REM sleep, whereas compounds of the present invention have the further advantage of only minimally disrupting REM sleep.

Summary Of The Invention

The present invention is directed to methods for inducing sleep in human beings. More particularly, the invention relates to the use of certain 3α-hydroxylated-5-reduced steroid derivatives and prodrugs thereof which act at a recently identified site on the $GABA_A$ receptor complex to treat insomnia.

Experiments by the inventors have established that the compounds used when practicing the invention give a rapid onset of sleep and have high potency and efficacy. They also cause less rebound insomnia and fewer effects on body temperature than known hypnotic drugs used to treat insomnia.

Brief Description Of The Drawings

These and other features, aspects, and advantages of the present invention will become better understood from the following description, the appended claims, and the accompanying drawings, where:

FIGS. IA and IB are plots of the percentage of NREM sleep per hour in rats vs. clock time for 30mg/kg and 3 & 10mg/kg of pregnanolone, respectively, administered at circadian time 18 hours (CT-18);

FIG. IC is a plot of the percentage of NREM sleep per hour in rats vs. clock time for 10mg/kg of pregnanolone, administered at CT-5.

FIGS. IIA and IIB are plots of the minutes spent sleeping vs. clock time when 30mg/kg and 3 & 10mg/kg of pregnanolone, respectively, administered at CT-18;

FIG. IIC is a plot of the minutes spent sleeping vs. clock time for 10mg/kg of pregnanolone administered at CT-5.

FIGS. IIIA and IIIB are plots of the mean deviation in body temperature vs. clock time for 30mg/kg and 3 & 10mg/kg of pregnanolone, respectively, administered at CT-18;

FIG. IIIC is a plot of the mean deviation in body temperature vs. clock time for 10mg/kg of pregnanolone administered at CT-5.

FIGS. IVA and IVB are plots of counts of movement per hour (locomotor activity) vs. clock time for 30mg/kg and 3 & 10mg/kg of pregnanolone, respectively, administered at CT-18;

FIG. IVC is a plot of counts of movement per hour (locomotor activity) vs. clock time for 10mg/kg of pregnanolone, administered at CT-5.

FIGS. VA and VB are plots of the percentage of REM sleep per hour vs. clock time for 30mg/kg and 3 & 10mg/kg of pregnanolone, respectively, administered at CT-18;

FIG. VC is a plot of the percentage of REM sleep per hour vs. clock time for 10mg/kg of pregnanolone, administered at CT-5.

FIGS. VIA, VIB, and VIC are plots if the percentage of NREM sleep per hour vs. clock time for 0.4 mg/kg of Triazolam administered at CT-5, for 0.4 mg/kg administered at CT-18, and for 0.4 mg/kg and 1.6 mg/kg of Triazolam administered at CT-18, respectively.

FIG. VID is a plot of the minutes spent sleeping vs. clock time for 0.4 mg/kg and 1.6 mg/kg Triazolam administered at CT-18.

FIG. VIE is a plot of the mean deviation in body temperature vs. clock time for 0.4 mg/kg and 1.6 mg/kg Triazolam administered at CT-18.

FIG. VIF is a plot of the counts of body movement per hour (locomotor activity) vs. clock time for 0.4 mg/kg and 1.6 mg/kg Triazolam administered at CT-18.

FIG. VIG is a plot of the percentage of REM sleep per hour vs. clock time for 0.4 mg/kg and 1.6 mg/kg of Triazolam administered at CT-18.

FIGS. VIIA and VIIB show EEG patterns during NREM sleep for 10 mg/kg of pregnanolone and for 30 mg/kg Zolpidem, respectively.

FIGS. VIIIA, VIIIB, and VIIIC are plots of the percentage of NREM sleep per hour vs. clock time for 10 mg/kg of Zolpidem administered at CT-5 and CT-18, respectively, and for 30 mg/kg of Zolpidem administered at CT-18.

FIG. VIIIC is a plot of the minutes spent sleeping vs. clock time for 10 mg/kg and 30 mg/kg Zolpidem administered at CT-18.

FIG. VIIID is a plot of the mean deviation in body temperature vs. clock time for 10 mg/kg and 30 mg/kg of Zolpidem administered at CT-18.

FIG. VIIIE is a plot of the counts of body movement per hour (locomotor activity) vs. clock time for 10 mg/kg and 30 mg/kg Zolpidem administered at CT-18.

FIG. VIIIF is a plot of the percent REM sleep per hour vs. clock time for 10 mg/kg and 30 mg/kg Zolpidem administered at CT-18.

FIGS. IXA and IXB are plots of the percentage of NREM sleep per hour vs. clock time for 40 ug/kg of dexmedetomidine administered at CT-5 and CT-18, respectively.

FIGS. XA, XB, and XC are plots of the percentage of total power (activity during NREM sleep) vs. minutes after treatment for pregnanolone, Zolpidem, and Triazolam, respectively, administered at CT-5.

FIGS. XIA, XIB, and XIC are plots of the percent of total power (activity during NREM sleep) vs. frequency for pregnanolone, Zolpidem, and Triazolam, respectively, administered at CT-5.

FIG. XII is a bar graph showing the change from baseline in locomotor activity for the first hour after treatment with various drugs. Locomotor activity is measured in counts per hour.

FIG. XIII is a bar graph depicting the change in REM sleep 2-3 hours after treatment with various drugs.

FIG. XIV shows plots of counts of movement per 5 minutes vs. minutes after treatment for pregnanolone and Zolpidem administered at CT-18.

FIGS. XV, XVI, XVII, and XVIII are plots of human plasma concentration of pregnanolone for Formulations 1, 2, 3, and 4, respectively. Arrows indicate time points when sleep was observed.

FIG. XIX is a plot of serum concentration in human subjects of pregnanolone versus time for all four formulations.

FIG. XX is a table showing human mean plasma concentrations of pregnanolone from two formulations at half-hour intervals.

FIG. XXI is a table showing the mean time awake in male human subjects during the 5 minute polygraphic recording sessions at half-hour intervals from two formulations of pregnanolone.

FIG. XXIIA is a graph plotting the number of sleep attempts by male human subjects verses plasma concentration of pregnanolone.

FIG. XXIIB is a table showing that the number of sleep attempts by male human subjects during a study day is related to the plasma concentration of pregnanolone.

FIG. XXIIIA-C are tables showing the mean change in NREM sleep, the mean change in REM rebound, and the mean change in REM sleep, respectively in rats receiving 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, 3α-hydroxy-3β-methyl-5α-pregnan-20-one, 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate sodium salt, 3α-hydroxy-3β-ethynyl-5α-pregnan-20-one, 3α,21-dihyrdoxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate, 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one, 3α-hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one, 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one 21-phosphate disodium salt, or controls.

FIGS. XXIVA and XXIVB are plots of the percentage of NREM sleep and REM sleep, respectively, per hour in rats vs. clock time for 18 mg/kg of 3β-ethynyl-3α-hydroxy-5β-

pregnan-20-one (CCD-3093), administered at circadian time 18 hours (CT-18).

FIG. XXVA is a plot of the minutes spent sleeping vs. clock time for 18 mg/kg of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one administered at CT-18.

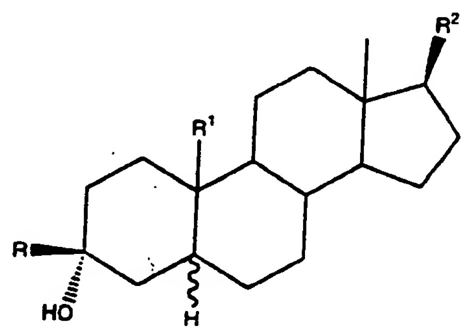
FIGS. XXVIA and XXVIB are plots of the mean deviation in body temperature vs. clock time and of counts of movement per hour (locomotor activity) vs. clock time, respectively, for 18 mg/kg of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one administered at CT-18.

Description

The compounds used in this invention are 3α-hydroxylated-5-reduced steroids which interact with the $GABA_A$ receptor complex. These compounds include various 3α-hydroxylated-5-pregnan-20-ones; 3α-hydroxylated-5-androstanes; and 3α,21-pregnanediol-20-ones; and ketone, ester, ether, sulfonate, sulfate, phosphonate, phosphate, oxime, and thiazolidine derivatives thereof, which derivatives are referred to as prodrugs. The expression "prodrug" denotes a derivative of a known direct acting drug, in this case a drug used in the invention, which derivative has enhanced delivery characteristics and therapeutic value as compared to the drug, which is transformed into the active drug by an enzymatic or chemical process in the patient's body; see Notari, R.E., "Theory and Practice of Prodrug Kinetics," Methods in Enzymology, 112:309-323 (1985); Bodor, N., "Novel Approaches in Prodrug Design," Drugs of the Future, 6(3):165-182 (1981); and Bundgaard, H., "Design of Prodrugs: Bioreversible Derivatives for Various Functional Groups and Chemical Entities," in Design of Prodrugs (H. Bundgaard, ed.), Elsevier, New York (1985). It should be noted that some of the synthetic derivatives useful in the method of the present invention may not be true prodrugs because, in addition to the above characteristics, they

also possess intrinsic activity. However, for present purposes they will be referred to as prodrugs.

Among the 3α-hydroxy pregnanes and 3α-hydroxy androstanes useful in the method of this invention are 3α-hydroxy-3β-R-5α or 5β-19-R^1-17β-R^2-pregnanes and 3α-hydroxy-3β-R-5α or 5β-19-R^1-17β-R^2-androstanes having the structural formula I:



I

wherein:

R is hydrogen, a lower alkyl group, e.g., methyl and methoxymethyl, a lower alkynyl group, e.g., ethynyl, 4-hydroxypent-1-ynyl and (4'-acetylphenyl)ethynyl, a lower trihaloalkyl group, e.g., trifluoromethyl, a lower monohaloalkyl group, e.g. chloromethyl, a lower alkenyl group, e.g., ethenyl and 2-phenylethenyl, an aryl group, e.g., phenyl, or an aralkyl group, e.g., benzyl;

R^1 is hydrogen or methyl;

R^2 is methylene (=CH_2), cyano, hydroxymethyl, methoxymethylene (=$CHOCH_3$), acetyl, 2'-hydroxyacetyl, 2'-hydroxyacetyl acetate, 1-hydroxyethyl, 2'-hydroxyacetyl hemisuccinate, 2'-methoxyacetyl, pyrid-4-ylthioacetyl, ethylene (=$CHCH_3$), propylene (=$CHCH_2CH_3$), 2'-hydroxyacetyl hemisuccinate sodium salt, 1-hydroxybutyl, and 1-hydroxy-1-methylethyl, 1-hydroxypropyl, 1-propionyl, 3-methoxypropionyl, ethynyl, 2'-mesyloxyacetyl, or 1'-(ethylenedioxy)ethyl;

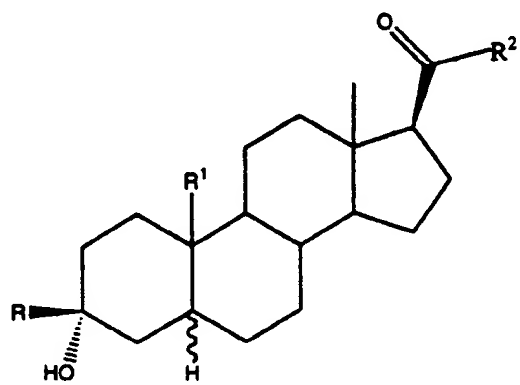
10/

and pharmaceutically acceptable 3-esters, 20-esters, 21-esters, 3,20-diesters, and 3,21-diesters, thereof;

with the proviso that the 3α-hydroxy-5α-pregnanes do not have a 21-hydroxy substituent.

A preferred group of compounds includes those in which R is not hydrogen, the compound has an IC_{50} value of ≤ 300nM as an allosteric modulator of [^{35}S] TBPS binding, and the compound is orally active.

Another preferred group of compounds which can be used in the method of this invention are 3α-hydroxy-3β-R-5α or 5β-19-R^1-21-R^2-pregnan-20-ones having the structural formula II:



II

wherein:

R is a lower alkyl group, e.g., methyl, a lower alkynyl group, e.g. ethynyl, or a trihalo(lower) alkyl group, e.g., trifluoromethyl;

R^1 is hydrogen or a lower alkyl group;

R^2 is hydrogen, hydroxy, a pyrid-4-ylthio group, a hemisuccinoyloxy group, or a phosphoryloxy group and the sodium salts thereof; with the provisos that:

R can not be H

(1) when R is hydrogen, R^2 is a pyrid-4-ylthio group;

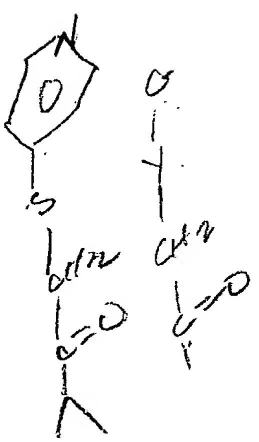
(2) when R^1 is hydrogen, R is a trihalo(lower) alkyl group; and

(3) when R^2 is other than hydrogen or a pyrid-4-ylthio group, R is a trihalo(lower) alkyl group.

A preferred group of compounds of Formula II is where R is a lower alkynyl group or a trihalo (lower alkyl) group.

Another preferred group of compounds of Formula II is where R^2 is a hemisuccinoyloxy group or a phosphoryloxy group, or sodium salts thereof.

The preferred neuroactive steroids of the present invention include 3α-hydroxy-5β-pregnan-20-one (pregnanolone) (available from Diosynth); 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt; 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one; 3α-hydroxy-3β-methyl-5α-pregnan-20-one; 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate sodium salt; 3α-hydroxy-3β-ethynyl-5α-pregnan-20-one; 3α,21-dihydroxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate; 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one; 3α-hydroxy-21(pyrid-4-ylthio)-5β-prenan-20-one; and 3α-hydroxy-3β-trifluoromethyl-5β-19-norpregnan-20-one 21-phosphate disodium salt.



Definitions

In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

The term "alkyl" refers to saturated aliphatic groups including straight chain, branched chain, and cyclic groups with 1 to 10 carbon atoms. Alkyl includes substituted lower alkyl.

The term "alkynyl" refers to unsaturated groups which contain at least one carbon-carbon triple bond and includes straight chain, branched chain, and cyclic groups. Alkynyl includes substituted lower alkynyl.

The term "trihaloalkyl" refers to alkyl groups substituted with three halogen atoms, F, Br, Cl or I. Preferred trihaloalkyl groups include trifluoromethyl and 2',2',2'-trifluoroethyl.

The term "lower" referred to herein in connection with organic radicals or compounds defines such as up to and including 8, preferably up to and including 6, and advantageously one, two, three, or four carbon atoms.

The term "alkenyl" refers to unsaturated groups which contain at least one carbon-carbon double bond and includes straight chain, branched chain, and cyclic groups. Alkenyl is meant to include the allenyl group, which possesses two consecutive double bonds, $>C=C=C<$. Alkenyl may be substituted with aryl.

The term "aryl" refers to aromatic groups which have at least one ring having a conjugated pi electron system and includes carbocyclic aryl and biaryl groups, all of which may be substituted.

"Carbocyclic aryl" groups are groups wherein the ring atoms on the aromatic ring are carbon atoms.

The term "biaryl" represents aryl groups substituted with other aryl groups.

The term "substituted lower alkyl" refers to lower alkyl groups substituted with an azido, cyano, or a lower alkoxy group.

"Alkoxy" refers to an alkyl group linked to a carbon atom through an oxygen ether-linkage.

The term "monohaloalkyl" refers to an alkyl group substituted with one halogen atom, F, Br, Cl, or I.

The term "substituted aryl" refers to aryl groups substituted by one to five substituents, independently selected from lower alkyl, aryl, hydroxy, lower alkoxy, amino, halo, lower acyl, nitro, lower trihaloalkyl, cyano, carboxy and lower acyloxyacetyl.

The term "amino" refers to -NRR′ where R and R′ are independently selected from hydrogen or lower alkyl, or it may refer to a morpholino group.

The term "halo" refers to the halogen atoms F, Br, Cl, and I.

The term "acyl" refers to -C(O)R where R is lower alkyl.

The term "carboxy" refers to the group -C(O)OH.

The term "acyloxyacetyl" refers to $-C(O)CH_2OC(O)R$ where R is lower alkyl.

The term "substituted lower alkynyl" refers to lower alkynyl groups substituted by one substituent selected from the group consisting of aryl, hydroxy, halo, and keto.

The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl and may be substituted on the aryl.

The term "pharmaceutically acceptable" refers to esters or salts derived from the combination of a steroid used in this invention and an organic or inorganic acid. Preferred esters of hydroxy groups at positions 3, 20 and/or 21 are those described by their corresponding acids: acetic, propionic, maleic, fumaric, ascorbic, pimelic, succinic, glutaric, bismethylene-salicylic, methanesulfonic, ethane-di-sulfonic, oxalic, tartaric, salicylic, citric, gluconic, itaconic, glycolic, p-aminobenzoic, aspartic, glutamic, gamma-amino-butyric, α-(2-hydroxyethylamino)-propionic, glycine and other α-amino acids, phosphoric, sulfuric, methane sulfonic, glucuronic, and 1-methyl-1,4-dihydronicotinic. Especially preferred are esters of acetic, succinic, and phosphoric acids.

It is believed that the substituent at the 3α-position can be either hydroxyl or any ester due to the fact that the ester will be cleaved off as the prodrug is converted to drug form. These are referred to herein as cleavable esters.

The following synthetic methods and examples are directed to the preparation of compound used in the present invention.

Synthetic Methods

The compounds according to the invention may be prepared by any convenient method, e.g. using conventional techniques such as are described in "Steroid Reactions" Djerassi, published in 1963 by Holden-Day, Inc., San Francisco or "Organic Reactions in Steroid Chemistry", Fried and Edwards, published in 1972 by Van Nostrand-Reinhold Co., New York.

The compounds of the invention may be prepared by any suitable technique known in the art or henceforth developed.

GENERAL METHODS

20-hydroxy pregnanes were prepared by the reduction 20-keto pregnanes with conventional reducing agents.

21-hemisuccinates were prepared from pregnan-20-one derivatives which were first brominated with molecular bromine to obtain the corresponding 21-bromo pregnanes. The bromo compounds were then reacted with various dioic acids, such as succinic acid, in the presence of an amine to yield 21-hydroxy esters. The resulting esters from the dioic acids were then converted to their sodium salts by conventional means.

The esters may be formed using reactions well known in the art between the hydroxyl group on the compounds discussed above with an organic and inorganic acid, acid halide, acid anhydride, or ester.

3β-Substituents

Halomethyl

The 3β-monohalomethyl compounds of this invention may be prepared by treatment of a 3-spiro-2'oxirane steroid with a source of halide ions in an inert solvent, such as tetramethylammonium halide in toluene, and preferably a proton source, such as acetic acid.

Saturated or unsaturated alkyl

Other 3-substituted steroids may be prepared by the addition of an organometallic reagent to a 3-ketosteroid in which other reactive functional groups may be protected as necessary. Thus, 3-alkynyl compounds may be prepared by the use of an lithium acetylide in inert solvents or with a reagent prepared *in situ* from 1,2-dibromoethylene and butyl lithium as the organometallic reagent. Likewise, compounds of Formula I in which R is alkenyl group may be prepared by the reaction of a 3-ketosteroid with a vinyl organometallic reagent such as vinyl magnesium bromide. Compounds in which the unsaturation is removed from the site of reaction, such as a 2-propenyl group, may also be prepared with reagents such as allyl magnesium bromide. Similarly, the use of an alkyl Grignard such as methyl magnesium iodide will lead to 3-alkyl compounds.

Trifluoromethyl

The-trifluoromethyl group may be prepared by reaction of a 3-ketosteroid with trimethyltrifluoromethylsilane catalyzed by fluoride ion.

21-Oxygenated compounds

Lead tetraacetate oxidation of 21-methyl

Various compounds of this type may be prepared by a reaction sequence in which a pregnan-20-one is oxidized with lead tetraacetate to give a 21-acetoxy derivative, hydrolysis of the acetate to give a 21-alcohol. The 21-alcohol may then be acylated with an appropriate carboxylic acid derivative, such as an acid anhydride or acid chloride or other reagent capable of replacing the hydrogen of the hydroxyl group, such as methanesulfonyl chloride.

Bromination of C-21

Alternatively, a pregnan-20-one may be treated with a brominating agent such as bromine in methanol to give a 21-bromopregnan-20-one. The bromide may then be displaced with an oxygen nucleophile, such as succinate anion, to give other 21-substituted pregnan-20-ones.

Pregnan-17-enes

These may be formed by the reaction of a 17-ketosteroid with a Wittig reagent such as the ylide derived from treatment of *n*-propyltriphenylphosphonium bromide with a strong base such as potassium *t*-butoxide.

3,20-Diols

A pregnan-3,20-diol may be formed by addition of a borane hydride such as diborane to the double bond of a pregnan-17-ene followed by oxidation of the thus formed organoborane with, for example, hydrogen peroxide, to give the 20-hydroxy. Alternatively, pregnan-3,20-diols may be formed by the reduction of a 20-one group to a 20-hydroxy group. Suitable reagents are hydride reagents such as sodium borohydride or dissolving metals such as sodium in n-propanol and the like.

EXAMPLE 1

a. 3α-Hydroxy-5β-pregnan-20-one cyclic 20-(1,2-ethanediyl ketal)

A mixture of 3α-hydroxy-5β-pregnan-20-one (10.8 g, 34 mmol), ethylene glycol (45 mL), and triethyl orthoformate (30 mL) was stirred at r.t. for 5 min. p-Toluene sulfonic acid (pTSA) (200 mg) was then added and the stirring was continued at r.t. for 1.5 hr. The resulting thick paste was poured into a sat. $NaHCO_3$ solution (250 mL). The precipitated solid was collected by filtration, washed thoroughly with cold water and dried. This semi-dried product was dissolved in CH_2Cl_2 (350 mL) and dried over

anhyd. K_2CO_3. The solution of the ketal was then filtered and used as such for the next step.

b. <u>5β-Pregnan-3,20-dione cyclic 20-(1,2-ethanediyl ketal)</u>

The above solution of 3α-hydroxy-5β-pregnan-20-one cyclic 20-(1,2-ethanediyl acetal) in CH_2Cl_2 was stirred with N-methylmorpholine-N-oxide (8.8 g, 75 mmol), and powdered 4Å molecular sieves (58 g) under N_2 for 15 min. Tetrapropylammonium perruthenate (400 mg) was then added and the stirring was continued at r.t. for 2 hr. The resulting dark green mixture was passed through a short column of Florisil and eluted with CH_2Cl_2. The fractions containing the product (by TLC) were combined and evaporated. The crude product was then crystallized from a mixture of EtOAc:Hex (1:1) to yield the title compound (10.3 g) as long rods.

An analogous method was used to prepare 5α-pregnan-3,20-dione cyclic 20-(1,2,ethanediyl ketal).

c. <u>3α-hydroxy-3β-phenylethynyl-5β-pregnan-20-one</u>

A solution of 5β-Pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (180 mg, 0.5 mmol) in dry THF (15 mL) was treated with lithium phenylacetylide (1M in THF, 1.5 mmol, 1.5 mL) at -70°. After stirring the mixture at this temp. for 1 h and then at r.t. for 2 h, it was quenched with 2N HCl (1 mL). The solvent was removed and the residue was dissolved in acetone (20 mL). After adding 1N HCl (5 mL) the solution was stirred at r.t. for 15 h. The solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (300 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. On elution with toluene:acetone mixture

(95:5) gave 3α-phenylethynyl-3β-hydroxy-5β-pregnan-20-one (70 mg) as a first fraction. Further elution with the same solvent mixture yielded 3β-phenylethynyl-3α-hydroxy-5β-pregnan-20-one (160 mg), m.p. 188-190°C.

EXAMPLE 2

a. <u>3α-hydroxy-3β-(2'-phenylethyl)-5β-pregnan-20-one</u>

A solution of 3α-hydroxy-3β-phenylethynyl-5β-pregnan-20-one (44 mg) was dissolved in EtOAc (12 mL), Pd/C (5%, 12 mg) was added and the mixture was hydrogenated at 400 Kpa pressure overnight at r.t. Filtration of the catalyst followed by evaporation of the solvent yielded the crude product, which was purified by chromatography over silica gel to isolate the pure title compound (33 mg); mp 153-154°C; TLC Rf (hexane:acetone 7:3) = 0.4.

EXAMPLE 3

a. <u>3β-(3',4'-Dimethoxyphenylethynyl)-3α-hydroxy-5β-pregnan-20-one</u>

A solution of 2,2-dibromo-1-(3',4'-dimethoxyphenyl) ethene (prepared by the Wittig reaction of 3,4-dimethoxybenzaldehyde with carbon tetrabromide in the presence of triphenyl phosphine) (966 mg, 3 mmol) in dry THF (15 mL) was treated under N_2 with n-BuLi (2.5M in THF, 6 mmol, 2.4 mL) at -78°. The mixture was stirred at this temp. for 2 hours and a solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (720 mg, 2 mmol) in dry THF (10 mL) was added dropwise over a period of 30 min. After stirring the resulting mixture at -78°C for 2 hr, the cooling bath was removed and the stirring was continued at r.t. for another hr. It was then quenched with 2N HCl solution (1 mL) at -10°C. The solvent was removed and the residue was then dissolved in acetone (25 mL). After adding 2N HCl (10 mL) the solution was stirred at r.t. for 2 h. A saturated $NaHCO_3$ soln. was added to neutralize the acid. The solvents were removed and the residue was

extracted with EtOAc. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (1.2 g). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (96:4) gave a phenylacetylene compound, which was not characterized. Further elution with the same solvent yielded 3α-(3',4'-dimethoxyphenylethynyl)-3β-hydroxy-5β-pregnan-20-one (120 mg) as a first fraction, and 3β-(3',4'-dimethoxyphenylethynyl)-3α-hydroxy-5β-pregnan-20-one as a second fraction (430 mg); mp 82-88°C.

An analogous method was used to prepare: 3β-(4'-methoxyphenylethnyl)-3α-hydroxy-5β-pregnan-20-one; 3β-(4'-Chlorophenylethynyl)-3α-hydroxy-5β-pregnan-20-one; 3β-(2'-Methoxyphenylethynyl)-3α-hydroxy-5β-pregnan-20-one; 3β-(4'-biphenylethynyl)-3α-hydroxy-5β-pregnan-20-one; 3β-(4'-dimethylaminophenylethynyl)-3α-hydroxy-5β-pregnan-20-one; 3β-(4'-Cyanophenylethynyl)-3α-hydroxy-5β-pregnan-20-one .

EXAMPLE 4

3β-(3',4'-Dimethoxyphenylethyl)-3α-hydroxy-5β-pregnan-20-one

A mixture of Pd/C (5%, 28 mg) and EtOAc (12 mL) was presaturated with hydrogen by stirring it under hydrogen for 10 min. A solution of 3β-(3',4'-dimethoxyphenylethynyl)-3α-hydroxy-5β-pregnan-20-one(185 mg) in EtOAc (5 mL) was then added and the mixture was hydrogenated at 300 Kpa pressure overnight at r.t. Filtration of the catalyst followed by evaporation of the solvent yielded the crude product, which was purified by chromatography over silica gel (hexane:acetone 4:1) to isolate the pure title compound (135 mg); TLC Rf (hexane:acetone 4:1) = 0.14.

EXAMPLE 5

3β-(4'-Nitrophenylethynyl)-3α-hydroxy-5β-pregnan-20-one

A solution of 2,2-dibromo-1-(4-nitrophenyl)ethene (prepared by the Wittig reaction of 4-nitrobenzaldehyde with carbon tetrabromide in the presence of triphenyl phosphine) (296 mg, 1 mmol) in dry THF (20 mL) was treated under N_2 with n-BuLi (2.5M in THF, 2 mmol, 0.8 mL) at -95°. The mixture was stirred at -80 to -100°C for 0.5 hr. and then a solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (720 mg, 2 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min. After stirring the resulting mixture at -80°C for 1 hr, and then at 0°C for 1 more hr. it was quenched with NH_4Cl solution (3 mL). The solvent was removed and the residue was then dissolved in acetone (25 mL). After adding 2N HCl (10 mL) the solution was stirred at r.t. for 1 h. Saturated $NaHCO_3$ soln. was added to neutralize the acid. The solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (400 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (96:4) gave the title compound as a brown solid (70 mg); TLC Rf (toluene:acetone 95:5) = 0.18.

EXAMPLE 6

3α-hydroxy-3β-phenyl-5β-pregnan-20-one

A solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (720 mg, 2 mmol) in dry THF was treated with Phenyl magnesium bromide (3M in THF, 6 mmol, 2 mL) at -70°C. After stirring the mixture at this temp. for 3 h and then at r.t. for 2 h, it was quenched with 2N HCl (10 mL). The solvent was removed and the residue was dissolved in acetone (20 mL). After adding 1N HCl (5 mL) the

solution was stirred at r.t. for 15 h. The solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (1.3 g). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (95:5) gave 3α-phenyl-3β-hydroxy-5β-pregnan-20-one (420 mg) as a first fraction. Further elution with the same solvent mixture yielded 3β-phenyl-3α-hydroxy-5β-pregnan-20-one (185 mg), m.p. 182-184°.

EXAMPLE 7

3α-hydroxy-3β-benzyl-5β-pregnan-20-one

A solution of benzyl magnesium bromide (2M in THF, 2 mmol, 1 mL) was diluted with THF (15 mL) and was treated dropwise with a solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (360 mg, 1 mmol) in dry THF (15 mL) at -60°. After stirring the mixture at this temperature for 1 h and then at r.t. for 15 h, it was quenched with 2N HCl (10 mL). The solvent was removed and the residue was dissolved in acetone (20 mL). After adding 1N HCl (5 mL) the solution was stirred at r.t. for 30 min. It was neutralized with 2N NaOH. The precipitated solid was collected by filtration, washed with water and dried to yield 3β-hydroxy-3α-benzyl-5β-pregnan-20-one (238 mg). The filtrate was extracted with EtOAc. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to give the crude product (160 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (95:5) gave 3β-hydroxy-3α-benzyl-5β-pregnan-20-one (40 mg) as a first fraction. Further elution with the same solvent mixture yielded 3α-hydroxy-

3β-benzyl-5β-pregnan-20-one (30 mg), which was crystallized from hexane:CH_2Cl_2 (4:1) as colorless rods (15 mg); m.p. 133-141°C; TLC Rf (toluene:acetone 9:1) = 0.5.

EXAMPLE 8

3β-(1-Heptynyl)-3α-hydroxy-5β-pregnan-20-one

A solution of 1-heptyne (0.327 mL, 2.5 mmol) in dry THF (15 mL) was treated with n-BuLi (2.5M in THF, 2.5 mmol, 1 mL) at -78°C. After stirring the mixture at this temperature for 1 hr, a solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (360 mg, 1 mmol) in dry THF (15 mL) was added and the mixture was stirred at -78°C for 1 hr. It was then quenched with 2N HCl solution (1 mL). The solvent was removed and the residue was then dissolved in acetone (10 mL). After adding 2N HCl (10 mL) the solution was stirred at r.t. for 1 hr. Saturated $NaHCO_3$ soln. was added to neutralize the acid. The solvents were removed and the residue was extracted with EtOAc. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (400 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (93:7) gave 3β-(1-heptynyl)-3α-hydroxy-5β-pregnan-20-one (260 mg) as a colorless solid; mp 121-123°C.

An analagous method was used to prepare: 3β-(1-Hexynyl)-3α-hydroxy-5β-pregnan-20-one; 3β-(1-Octynyl)-3α-hydroxy-5β-pregnan-20-one; 3α-Hydroxy-3β-[6-oxo-1-heptynyl]-5β-pregnan-20-one, using 1-heptyne-6-one cyclic (1,2-ethanediyl acetal).

EXAMPLE 9

3β-[3'(R/S)-Hydroxybutynyl]-3α-hydroxy-5β-pregnan-20-one

A solution of 3(R/S)-hydroxybutyne (0.470 mL, 6 mmol) in dry THF (15 mL) was treated with n-BuLi (2.5M in THF,

12 mmol, 4 mL) at -70°C. After stirring the mixture at this temperature for 0.5 hr, a solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (1.08 g, 3 mmol) in dry THF (30 mL) was added and the mixture was stirred at -78°C for 1 hr. The cooling bath was removed and the mixture was stirred at r.t. for another 1.5 hr. It was then quenched with NH_4Cl solution (3 mL). The solvent was removed and the residue was then dissolved in acetone (10 mL). After adding 2N HCl (5 mL), the solution was stirred at r.t. for 1 hr. Saturated $NaHCO_3$ soln. was added to neutralize the acid. The solvents were removed and the residue was extracted with EtOAc. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (1.4 g). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (85:15) gave 3β-[3'(RS)-hydroxy-butynyl]-3α-hydroxy-5β-pregnan-20-one (145 mg) as a colorless solid; TLC Rf (toluene:acetone 4:1) = 0.24.

An analogous method was used to prepare: 3β-[4'(R/S)-hydroxypentynyl]-3α-hydroxy-5β-pregnan-20-one.

EXAMPLE 10

3β-(4'-Acetylphenylethynyl)-3α-hydroxy-5α-pregnan-20-one

A solution of 4-iodoacetophenone (95 mg, 0.39 mmol), 3β-ethynyl-3α-hydroxy-5α-pregnan-20-one (106 mg, 0.3 mmol) in dry degassed pyrrolidine (3 mL) was stirred under argon at r.t. Bis(triphenylphosphine)palladium chloride (5 mg) and CuI (5 mg) were added and the mixture was stirred at r.t. for 15 hr. The TLC showed 100% conversion of the starting material, hence, the mixture was quenched with NH_4Cl solution (15 mL) and was extracted with EtOAc. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the

solution was filtered and evaporated to yield the crude product (150 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with hexane:acetone mixture (4:1) afforded 3β-(4'-acetylphenylethynyl)-3α-hydroxy-5α-pregnan-20-one (35 mg) as a colorless solid; TLC Rf (hexane:acetone 7:3) = 0.4.

An analogous method was used to prepare: 3α-Hydroxy-3β-(4'-trifluoromethylphenylethynyl)-5β-pregnan-20-one; 3α-Hydroxy-3β-(4'-methylphenylethynyl)-5β-pregnan-20-one; 3α-Hydroxy-3β-(4'-acetoxyacetylphenylethynyl)-5β-pregnan-20-one; 3α-Hydroxy-3β-(pentafluorophenyl)ethynyl-5β-pregnan-20-one; 3β-(2'-Hydroxyphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one; 3β-(3'-Hydroxyphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one; and 3α-Hydroxy-3β-(4'-carboxyphenylethynyl)-5β-pregnan-20-one, ethyl ester.

EXAMPLE 11

3α-Hydroxy-3β-methoxymethyl-5α-pregnan-20-one

The 3(R)-epoxy-5α-pregnan-20-one was prepared by the reaction of 5α-pregnan-3,20-dione with trimethylsulfoxonium methyliodide in the presence of a strong base, such as potassium t-butoxide, in THF.

To a solution (there were few undissolved solid particles) of 3(R)-epoxy-5α-pregnan-20-one (5.145 g, 15.57 mmol) in CH_3OH (600 mL) was added freshly cut Na (~ 600 mg). CAUTION: H_2 gas evolution. After all Na had reacted, a clear solution resulted. The reaction solution was stirred overnight. Since TLC (3:1 hexane/acetone) indicated mainly the starting material, the mixture was heated (close to boiling point) for 8 h. and then left at room temperature overnight. TLC still showed a small amount of the starting material, besides two new, less-polar spots. Therefore, the mixture was heated for another 8 h. at which time there was no unreacted starting epoxide. After the reaction solution had come to room

temperature, glacial acetic acid (3 mL) was added dropwise. The solvent was then removed under reduced pressure, and CH_2Cl_2 and water were added. The aqueous layer was back-extracted with CH_2Cl_2.. The combined organics were washed with saturated $NaHCO_3$, dried (MgSO4), filtered, and evaporated under reduced pressure to give a white solid (5.55 g), which was found to be a 84:16 mixture of 3α-hydroxy-3β-methoxymethyl-5α-pregnan-20-one and its 17-epimer on the basis of its ^{1}H NMR. R-ecrystallization from hot 1:1 hexane/acetone furnished only 3α-hydroxy-3β-methoxymethyl-5α-pregnan-20-one (3.270 g, 58%) as white crystals. mp 163-4 °C. As expected, the mother liquors contained both 17-epimers in approximately equal amounts.

An analogous method was used to prepare: 3α-hydroxy-3β-ethoxymethyl-5α-pregnan-20-one; 3α-hydroxy-3β-propoxymethyl-5α-pregnan-20-one;

EXAMPLE 12

3α-hydroxy-3β-cyanomethyl-5α-pregnan-20-one

Potassium cyanide (210mg; 3.2mmol) and 20,20-ethylenedioxy-3*R*-spiro-2'-oxirane-5α-pregnan-20-one (1.0 g; 2.7mmol) were stirred in ethanol (175mL) for 2 days, refluxed for 5 hours and then allowed to cool overnight. Ether (100mL) and brine (100mL) were added to the reaction mixture. The separated aqueous phase was washed with ether and the combined organic phases were washed with brine. Aqueous HCl (1M; 6mL) and THF (15mL) were added to the organic phases and the solution stirred overnight. Aqueous $NaHCO_3$ (20mL) was added and then water (50mL). The separated aqueous phase was washed with ether and the combined organic phases were dried over $MgSO_4$ and evaporated. The white solid residue was purified by flash column chromatography (40:20:1 CH_2Cl_2/hexane/acetone) to give a white solid (493mg; 52%); mp 218.5 - 220° C.

EXAMPLE 13

3α-Hydroxy-3β-azidomethyl-5α-pregnan-20-one

To a solution of 3(R)-epoxy-5α-pregnan-20-one (1.012 g, 3.06 mmol) in dry DMF (Aldrich; 50 mL) was added NaN_3 (226 mg, 3.48 mmol), all of which did not dissolve. Then glacial acetic acid (1.05 mL, 17.5 mmol) was added, and the resulting mixture was heated a little bit with a heat gun in order to dissolve all the solid. The mixture was stirred for a while, giving a clear solution. TLC (100:1 CH_2Cl_2/acetone or 3:1 hexane/acetone) after stirring the mixture for 4 h still indicated the presence of starting material. The reaction mixture was then heated with stirring at 35-50 °C for 3 days. By TLC the reaction was complete. CH_2Cl_2 and water were added. The aqueous layer was back-extracted with CH_2Cl_2. The combined organics were washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated on rotary evaporator to give a liquid containing a lot of DMF. Further Evaporation by distillation under high vacuum provided a solid (1.09 g). ^1H NMR of this solid revealed the presence of mainly the desired 3α-hydroxy-3β-azidomethyl-5α-pregnan-20-one and almost none of its 17-epimer. Purification by flash column chromatography using CH_2Cl_2 as eluant afforded 3α-hydroxy-3β-azidomethyl-5α-pregnan-20-one (834 mg, 73%) as a white solid; mp 147-8°C.

EXAMPLE 14

3α,20α-Dihydroxy-5α-19-nor-pregnane

To a solution of 3α-hydroxy-5α-19-nor-17(Z)-pregn-17(20)-ene (232 mg, 0.81 mmol) (prepared from the androstane-17-one with the Wittig reagent as described below) in THF (20 mL) was added diborane THF complex (1M solution in THF, 3.0 mL) dropwise at 0°C. The reaction was allowed to warm to 25°C for 2.5 h. Then a solution of sodium hydroxide (2N, 15 mL) was added very slowly at 0°C followed by an addition of hydrogen peroxide (30%, 8 mL).

Water then was added and THF layer was separated by separatory funnel. The aqueous layer was extracted with ethyl acetate (2x40 mL). The organic solution was dried over potassium carbonate and the solvent removed in vacuo. A column chromatography using acetone and methylene chloride in hexane (8:8:84) resulted in a small amount of pure product (15mg).

EXAMPLE 15

3α,21-hydroxy-3β-methyl-5α-pregnan-20-one, 21-hemisuccinate

A mixture of 3α,21-hydroxy-3β-methyl-5α-pregnan-20-one (600 mg, 1.72 mmol) and succinic anhydride (388 mg, 3.88 mmol) in 12 mL of pyridine was allowed to stir at rt for 23h. The reaction was concentrated in vacuo and the residue was partioned between EtOAc and an aqueous HOAc solution (pH 4). The organic layer was separated and washed with a sat. NaCl solution and concentrated. The crude hemisuccinate was purified by flash chromatography (12 cm of silica in a 3.5 cm dia. column, eluted with 400 mL of 45/55 EtOAc/CH_2Cl_2 and 200 mL each of 50/50 and 60/40 EtOAc/CH_2Cl_2. Recrystallization from MeOH/water gave 346 mg (45%) of the hemisuccinate as a white solid.

The sodium salt of the hemisuccinate was prepared by dissolving the acid in methanol and adding one equivalent of an aqueous solution of sodium bicarbonate. The solvent was removed in vacuo and the residue was triturated with ether/pet. ether to give a white solid.

EXAMPLE 16

a. Triphenylprop-2-ynylstannane

Propargyl bromide (80 w% solution in toluene; 1.7 mL, 11.43 mmol) was added to a mixture of Mg (471 mg, 19.38 mmol) and dry Et_2O (25 mL). $HgCl_2$ (a few crystals) was then added. The mixture was stirred for ~5 min. Since no reaction occurred, more $HgCl_2$ (15 mg) was added. The

resulting mixture was first stirred at room temperature and then warmed briefly. The mixture became turbid, and the reaction ensued. The mixture was stirred at <20°C (cold water bath) for ~1 h after which time most of Mg had reacted. The amount of the unreacted Mg remained unchanged over a period of ~30 min. Therefore, propargyl bromide (80 w% in toluene; 0.4 mL, 2.69 mmol) was added. The mixture was stirred at <20°C for ~1 h. Subsequently, a suspension of triphenyltin chloride (95%; 5.045 g, 12.43 mmol) in Et_2O (50 mL) was added dropwise through a cannula to the above solution of propargylmagnesium bromide. The resulting mixture, which contained a white solid, was stirred at room temperature for ~3 h. TLC (3:1 hexane/acetone) showed complete consumption of the starting tin chloride. The mixture was quenched with saturated aqueous NH_4Cl (10 mL). A white solid was formed. The organic layer was separated from the aqueous layer that contained the white solid. Finally, the organic phase was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white solid (4.78 g). Recrystallization of the solid from hot hexane (50 mL) furnished triphenylprop-2-ynylstannane as white plate-shaped crystals (3.92 g, 81%).

b. <u>3α-Hydroxy-3β-(butadi-2',3'-enyl)-5α-pregnan-20-one</u> (Reference: Baldwin, J. E.; Adlington, R. M.; Basak, A. *J. Chem. Soc., Chem. Commun.* 1984, 1284)

A mixture containing 3β-iodomethyl-3α-hydroxy-5α-pregnan-20-one (234 mg, 0.51 mmol), triphenylprop-2-ynylstannane (795 mg, 2.04 mmol), azobisisobutylnitrile (18 mg, 0.11 mmol), and dry benzene (2 mL) was degassed for ~10 min. The above mixture was then refluxed for 2 h. As indicated by TLC (100:1 CH_2Cl_2/acetone), there was still some (~33%) unreacted starting material, the amount of which remained unchanged over a period of 2 h. TLC showed, besides the starting material, two spots in ~1:1 ratio, the less-polar spot being a new spot while the

other corresponding to 3β-methyl-3α-hydroxy-5α-pregnan-20-one. The reaction mixture was cooled to room temperature. After removing the solvent, the residue was subjected to flash chromatography to afford 3α-hydroxy-3β-(butadi-2',3'-enyl)-5α-pregnan-20-one as a white solid (20 mg, 11%).

EXAMPLE 17

3α-Hydroxy-3β-(prop-2'-ynyl)-5α-pregnan-20-one

To a mixture of Mg (268 mg, 11.02 mmol), propargyl bromide (80 w%; 1.5 mL, 10.09 mmol) and dry Et_2O (15 mL) was added $HgCl_2$ (10 mg). The resulting mixture was slightly warmed in a water bath in order to initiate the reaction. The mixture became turbid, and the reaction ensued. The mixture was stirred at <20°C until all Mg reacted (for ~1 h).

A solution of 20,20-ethylenedioxy-5α-pregnan-3-one (711 mg, 1.97 mmol) in dry Et_2O (20 mL) and dry THF (10 mL) was cooled to -78°C. Some of the starting material precipitated on cooling. Subsequently, propargylmagnesium bromide solution (~0.6 M; 6.5 mL), prepared above, was added dropwise at -78°C. The resulting mixture was stirred at -78°C for ~1 h. The mixture was then allowed to warm to room temperature where it was stirred for 1 h. 1N HCl, *p*-toluenesulfonic acid monohydrate, and acetone were added in order to hydrolyze the ketal function. The two-phase mixture was stirred at room temperature overnight. TLC (7:1 hexane/acetone) showed completion of the reaction. Et_2O and water were added. The aqueous layer was back-extracted with Et_2O. The combined organics were washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a white solid (690 mg). ^1H NMR of the crude solid indicated it to be a 1:2 mixture of the desired 3α-hydroxy-3β-(prop-2'-ynyl)-5α-pregnan-20-one and its 3-epimer, respectively. The mixture was flash-chromatographed twice with 10:1

hexane/acetone to provide 3α-hydroxy-3β-(prop-2'-ynyl)-5α-pregnan-20-one as a white solid (70 mg, 11%). m.p. 220-225°C (dec.).

EXAMPLE 18

3α-Hydroxy-3β-(but-3'-enyl)-5α-pregnan-20-one

(Reference: Keck, G. E.; Yates, J. B. *J. Am. Chem. Soc.* 1982, *104*, 5829)

Dry benzene (3 mL) was degassed for 15 min by bubbling Ar through it. Next were added successively 3β-iodomethyl-3α-hydroxy-5α-pregnan-20-one (263 mg, 0.574 mmol), allyltributyltin (97%; 0.5 mL, 1.56 mmol), 1,2'-azobisisobutyronitrile (20 mg, 0.122 mmol). The resulting mixture was refluxed for 3.5 h. TLC (100:1 CH_2Cl_2/acetone) showed complete consumption of the starting iodide derivative, with the appearance of two new spots in ~1:1 ratio, the more-polar spot corresponding to 3β-methyl-3α-hydroxy-5α-pregnan-20-one. The reaction mixture was cooled to room temperature. After removing the solvent, the residue was subjected to flash chromatography twice, with CH_2Cl_2 and 7:1 hexane/acetone. 3α-Hydroxy-3β-(but-3'-enyl)-5α-pregnan-20-one was obtained as a white solid (75 mg, 35%). m.p. 140-141°C.

EXAMPLE 19

a. 3α-Hydroxy-5β-pregnan-20-one, 20-ketal

A mixture of 3α-hydroxy-5β-pregnan-20-one (10.8 g, 34 mmol), ethylene glycol (45 mL), and triethyl orthoformate (30 mL) was stirred at r.t. for 5 min. p-toluenesulfonic acid (200 mg) was then added and the stirring was continued at r.t. for 1.5 hr. The resulting thick paste was poured into a sat. $NaHCO_3$ solution (250 mL). The precipitated solid was collected by filtration, washed thoroughly with cold water and dried. This semi-dried product was dissolved in CH_2Cl_2 (350 mL) and dried over anhyd. K_2CO_3.

The solution of the ketal was then filtered and used as such for the next step.

b. 5β-Pregnan-3,20-dione, 20-ketal

The above solution of 3α-hydroxy-5β-pregnan-20-one, 20-ketal in CH_2Cl_2 was stirred with N-methylmorpholine-N-oxide (8.8 g, 75 mmol), and powdered 4Å molecular sieves (58 g) under N_2 for 15 min. Tetrapropylammonium perruthenate (400 mg) was then added and the stirring was continued at r.t. for 2 hr. The resulting dark green mixture was passed through a short column of Florisil and eluted with CH_2Cl_2. The fractions containing the product (TLC) were combined and evaporated. The crude product was then crystallized from a mixture of EtOAc:Hx (1:1) to yield the title compound (10.3 g) as long rods.

c. Preparation of the lithium reagent from 1,2-dibromoethylene

A 100 mL three neck flask equipped with a N_2 gas bubbler, a thermometer, and a dropping funnel was charged with 1,2-dibromoethylene (cis/trans mixture, 98%, Aldrich, 0.164 mL, 2 mmol, mw = 186, d = 2.246). Dry THF (15 mL) was added and the solution was cooled to -78°C in a dry ice-acetone bath. n-BuLi (2.5M in THF, 1.6 mL, 4 mmol) was added dropwise over a period of 10 min. The mixture was stirred at this temperature for 1 hr and the resulting reagent was used immediately for the next step.

d. 3β-Ethynyl-3α-hydroxy-5β-pregnan-20-one

The above solution of the reagent in THF, which was maintained at -78°C, was treated dropwise with a solution of 5β-pregnan-3,20-dione, 20-ketal (180 mg, 0.5 mmol) in THF (15 mL). The temperature was maintained below -70°C during the addition. The stirring was continued at this temperature for 15 min. (100% conversion as detected by TLC). The cooling bath was removed and the resulting

solution was quenched with 2N HCl (pH 6). The solvent was removed and the residue was dissolved in acetone (10 mL). After adding 2N HCl (4 mL) the solution was stirred at ambient temperature for 0.5 h. The mixture was neutralized with dil. $NaHCO_3$ solution. The precipitated solid (158 mg, 93%) was collected by filtration, washed with water, and dried. The crude product is then purified by crystallization from EtOAc or from a mixture of acetone-hexane to yield the title compound; mp 196-197°C, TLC-R_f 0.45 (hexane:acetone 7:3).

e. <u>3β-Ethyl-3α-hydroxy-5β-pregnan-20-one</u>

A mixture of Pd/C (5%, 24 mg) and MeOH (12 mL) was presaturated with hydrogen by stirring the mixture under hydrogen for 10 min. A solution of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one(155 mg) in MeOH (5 mL) was then added, and the mixture was hydrogenated for 1.5 h. under 200 Kpa pressure at r.t. Filtration of the catalyst followed by evaporation of the solvent yielded the title compound, which was purified by crystallization from hexane:acetone (9:1); yield : 105 mg; mp 115-117° C. TLC Rf (hexane:acetone 7:3) = 0.48.

f. <u>3β-Ethyl-3α-hydroxy-5α-pregnan-20-one:</u>

To a mixture of 570 mg (3 mmol) of cuprous iodide and 10 ml of dry ether cooled to -60° C under argon was added 4 ml of 1.5M methyl lithium. The reaction mixture was warmed to 0° C, stirred until a colorless solution was obtained and then recooled to -60° C. An additional 0.5-1 ml of methyl lithium solution was added to keep the solution colorless. A cooled (0° C) solution of 0.5 g (1.5 mmol) of 3*R*-spiro-2'-oxirane-5α-pregnan-20-one in 20 ml of ether was added slowly to the above reaction mixture while maintaining the temperature at -60° C. An additional 0.2 ml of methyl lithium was added and the reaction mixture was stirred at -60° C for 1 hr. It was then quenched by the

addition of saturated ammonium chloride solution, allowed to warm to room temperature and diluted with ether and ammonium chloride solution. The organic phase was separated. The aqueous phase was extracted with ether. The combined organic extracts were dried and concentrated to leave 450 mg of crude product. Chromatography on silica gel using 5% acetone/hexane gave 356 mg of product.

EXAMPLE 20

Disodium 3α,20β-dihydroxy-5β-pregnane, bis(hemisuccinate)

A suspension of 3α,20β-dihydroxy-5β-pregnane (Steraloids; 250 mg, 0.78 mmol) in 5 mL of dry pyridine was treated with succinic anhydride (200 mg, 2.0 mmol). The mixture was heated at 100°C for a total of 10 h. An additional 6 mmol of succinic anhydride was added in three portions as the reaction was heated. The dark mixture was concentrated (0.05 mmHg, 30°C) to remove the solvent and then heated at 90°C (0.05 mmHg) to remove excess succinic anhydride. The residue was recrystallized from ether/hexanes giving a solid that consisted mainly of succinic acid. The mother liquor was concentrated and subjected to column chromatography (flash silica gel, eluted with 95/5/0.1 CH_2Cl_2/MeOH/HOAc) giving a white solid which was recrystallized from ether/hexane. The bis(hemisuccinate), mp 81-90°C was carried on to the bis(sodium salt).

The bis(hemisuccinate) (100 mg, 0.192 mmol) was dissolved in a minimum volume of methanol. A solution of $NaHCO_3$ (2 eq, 33 mg, 0.393 mmol) in 0.6 mL of water was added dropwise. After 3h, the solution was concentrated in vacuo to give a white solid.

EXAMPLE 21

a. 3α-Hydroxy-3β-methyl-5α-androstane 17β-carboxylic acid

Bromine (2.0 mL, 38.8 mmol) was added dropwise to an aqueous solution of NaOH (3.746 g in 30 mL of water) at 0°C. Subsequently, a solution of 3α-hydroxy-3β-methyl-5α-

pregnan-20-one in dioxane (250 mL) and water (30 mL) was added to the above solution at 0°C over a period of 1 h. The resulting yellow mixture that contained some solid was allowed to warm to room temperature. After some time all the solid dissolved. The reaction solution was stirred overnight at which point it turned colorless. Then CH_2Cl_2 (150 mL), water (100 mL) and 1N HCl (50 mL) were added. Brine was also added to facilitate the separation of the two liquid phases. The aqueous layer was separated and back-extracted with CH_2Cl_2 (200 mL). The two organic phases were combined and washed with water, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to afford a white solid (3.851 g). Recrystallization of this solid from hot 1:1 hexane/acetone (500 mL) gave 3α-hydroxy-3β-methyl-5α-androstane 17β-carboxylic acid as a white solid (1.838 g, 50%). m.p. 222.5-224°C. Evaporation of the mother liquor followed by recrystallization from hot methanol furnished more product as white crystals (1.50 g, 40%). m.p. 223-225°C.

b. 17β-(Hydroxymethyl)-3β-methyl-5α-androstan-3α-ol

To a stirred suspension of $LiAlH_4$ (95%; 546 mg, 13.67 mmol) in dry THF (30 mL) at 0°C was added a solution of 3α-hydroxy-3β-methyl-5α-androstane 17β-carboxylic acid (1.633 g, 4.88 mmol) in dry THF (50 mL + 10 mL for the rinse) through a pressure-equalizing funnel over a period of 30 min. The resulting mixture was then allowed to warmed to room temperature. The stirring was continued overnight. TLC (7:1 hexane/acetone) showed completion of the reaction. The reaction mixture was cooled to 0°C, and 1N HCl (25 mL) was added dropwise. Subsequently, Et_2O (100 mL), water (25 mL), and conc. HCl (5 mL) were added. The aqueous layer was still not completely clear. The two phases were separated. Conc. HCl (15 mL) was added to the aqueous layer which was then extracted with Et_2O (100 mL). The two organic layers were combined, washed with saturated aqueous

$NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to afford 17β-(hydroxymethyl)-3β-methyl-5α-androstan-3α-ol as a white solid (1.605 g). ^1H NMR of the product showed it to contain some (4%) unreacted starting material and THF (4%).

c. <u>17β-Formyl-3β-methyl-5α-androstan-3α-ol</u>

To a solution of 17β-(hydroxymethyl)-3β-methyl-5α-androstan-3α-ol (96% pure; 1.58g, 4.73 mmol) in dry CH_2Cl_2 (90 mL) at room temperature was added pyridinium chlorochromate (98%; 1.115 g, 5.07 mmol). The resulting mixture was stirred at room temperature for 7 h. TLC (7:1 hexane/acetone) showed complete consumption of the starting acid. The mixture was then filtered through neutral florisil and successively washed with CH_2Cl_2 (250 mL) and 5:1 hexane/acetone (100 mL). (The filtrate was checked by TLC for the presence of the product.) The solvents were removed to give a light yellow solid (1.390 g) which was purified by flash chromatography employing CH_2Cl_2 as eluent to furnish 17β-formyl-3β-methyl-5α-androstan-3α-ol as a white solid (1.081 g, 72%). m.p. 156-166°C.

d. <u>17β-cyano-3α-hydroxy-5α-androstane</u>

To a solution of 3α-hydroxy-5α-androstan-17-one (1.511 g, 5.20 mmol) in 135 mL of 1,2-dimethoxyethane (DME) was added a 1M solution of potassium *tert*-butoxide in THF (52 mL, 52 mmol). The resulting cloudy orange solution was treated over 2.5 h. with a solution of 2.06 g (10.6 mmol) of *p*-toluenesulfonylmethyl isocyanide in 30 mL of DME. After an additional 2.5 h., the reation was diluted with 100 mL of ether and 100 mL of water was added. The aqueous layer was extracted with ether (2 x 50 mL) and the pooled organic layers were washed with a brine solution, dried ($MgSO_4$) and concentrated. Column chromatography on silica gel (100% of CH_2Cl_2 as eluent) gave 860 mg of slightly

impure product. Recrystallization from EtOAc gave 300 mg (19%) of pure nitrile, mp 159-160°C.

EXAMPLE 22

3α-hydroxy-5β-pregn-17(20)-ene

To the ylide prepared from ethyltriphenylphosphonium bromide (Aldrich; 1.606 g, 4.33 mmol) and solid potassium *tert*-butoxide (475 mg, 4.23 mmol) was added 3α-hydroxy-5β-androstan-17-one (Steraloids; 503 mg, 1.73 mmol) in one portion. The red-orange mixture was heated at reflux for 3.5 h and then allowed to cool. It was further cooled to 0°C and then added to an ice-cold sat. NH_4Cl solution/ether mixture. The aqueous layer was separated and washed twice with ether. The ether layers were then extracted with a sat. NaCl solution, dried ($MgSO_4$) and concentrated. Flash chromatography (17.5 cm of silica in a 4 cm dia. column, eluted with 400 mL of 2% acetone/CH_2Cl_2 and 200 mL of 2.5% acetone/CH_2Cl_2) afforded 530 mg (100%) of the alkene as a white solid. By ^{1}H NMR the compound was an 8:1 mixture of *Z*- and *E*-isomers.

EXAMPLE 23

3α-hydroxy-3β-ethenyl-5β-pregnan-20-one

A solution of 5β-pregnan-3,20-dione, 20-ketal (1.18g, 3.3 mmol) in dry THF (20 mL) was treated with vinyl magnesium bromide (1M in THF, 3.7 mmol, 3.7 mL) at -70°. After stirring the mixture at this temp. for 5 min. and then at r.t. for 2.5 h, it was quenched with sat. NH_4Cl solution (10 mL). The solvent was removed and the residue was extracted with EtOAc. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (1.2 g). This crude product was then dissolved in acetone (20 mL). After adding 1N HCl (10 mL) the solution was stirred at r.t. for 15 h. The solvents were removed and the residue was

extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (890 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (94:6) gave 3α-ethenyl-3β-hydroxy-5β-pregnan-20-one (126 mg) as a first fraction. Further elution with the same solvent mixture yielded 3β-ethenyl-3α-hydroxy-5β-pregnan-20-one (189 mg), m.p. 113-116°.

EXAMPLE 24

a. 3α-Hydroxy-5α-androstan-17-one

To a solution of 3β-hydroxy-5α-androstan-17-one (6g) and diethyl azodicarboxylate (5.04 g) in THF (50 mL) was added trifluoroacetic acid (3.3 g) and the mixture became yellow. Then triphenylphosphine (7.6 g) was added. The reaction turned to colorless and heat was given off. Sodium benzoate was added after 5 min and then water (100 mL) was added. The mixture was extracted with methylene chloride (3x80 mL) and organics was dried over magnesium sulfate. The solvent was removed in vacuo and the crude product was hydrolyzed with potassium hydroxide (10%, 10 mL) in methanol (150 mL) for 1 h. Most of the methanol was then removed in vacuo and the remaining material was partitioned between methylene chloride and ammonium chloride. The product (4.7 g, 78%) was purified by chromatography (CH_2Cl_2: acetone=9:1).

b. 3α-Hydroxy-21-methyl-5α-pregn-17(Z)-ene

3α-Hydroxy-5α-androstan-17-one (2 g, 6.9 mmol) was added to Wittig reagent produced from propyltriphenyl-phosphonium bromide (13.3 g) and potassium *t*-butoxide (3.9 g) in THF (20 mL). The reaction was refluxed for 12 h and cooled to 25°C. Then a solution of ammonium chloride (60 mL) was added and the organic layer was separated by

separatory funnel. The aqueous layer was extracted with methylene chloride (2x50 mL). The organic solution was dried over potassium carbonate and the solvent removed in vacuo. The crude product was purified by chromatography (acetone:methylene chloride:hexane = 1:2:7) to produce 0.84 g of product (39%). It was mixture of Z and E isomers (Z:E=13:1).

An analogous procedure was used to prepare 3α-hydroxy-17-methylene-5α-androstane using the Wittig reagent produced from methyltriphenylphosphonium bromide.

c. <u>3α-t-Butyldimethylsiloxy-21-methyl-5α-pregn-17(Z)-ene</u>

The mixture of 5α-3α-hydroxy-21-methyl-pregn-17(Z)-ene (0.84 g, 2.66 mmol), TBDMSCl (1.2 g, 8.0 mmol) and imidazole (0.91 g, 13.3 mmol) in methylene chloride (10 mL) and DMF (30 mL) was stirred for 12 h and then ammonium chloride was added. It was then extracted with methylene chloride (3x40 mL) and washed with brine (50 mL). The organic solution was dried over potassium carbonate and then solvent removed and it was found that there was still some DMF. It was then redissolved in ether and washed with brine (2x50 mL) and then dried over potassium carbonate. Chromatography with hexane resulted 1.14 g of the pure product (100%).

d. <u>3α-t-Butyldimethylsiloxy-20α-hydroxy-21-methyl-5α-pregnane</u>

To a solution of 3α-t-butyldimethylsiloxy-21-methyl-5α-pregn-17(Z)-ene (1.14 g, 2.66 mmol) in THF (30 mL) was added diborane THF complex (1M solution in THF, 5.3 mL) dropwise at 0°C. The reaction was allowed to warm to 25°C for 1 h. Then a solution of sodium hydroxide (20%, 10 mL) was added very slowly at 0°C followed by an addition of hydrogen peroxide (30%, 10 mL). Aqueous ammonium chloride then was added and THF layer was separated by separatory funnel. The aqueous layer was extracted with ether (2x40

mL). The organic solution was dried over potassium carbonate and the solvent removed in vacuo. The pure product was obtained by column chromatography (0.52 g, 44%).

e. 3α,20α-Dihydroxy-21-methyl-5α-pregnane

A solution prepared by mixing HF (48%, 5 mL) and CH_3CN (30 mL) was added to a flask containing 3α-t-butyldimethylsiloxy-20α-hydroxy-21-methyl-5α-pregnane (0.51 g) and a white precipitate appeared. The reaction was stirred for 1 h and then filtered. The white solid was washed with ether for three times and it gave satisfactory analytical results (0.3 g, 79%). m.p. 227-231°C.

EXAMPLE 25

3α,21-Dihydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one:

To a solution of 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one (300 mg, 0.87 mmol) in toluene (15 mL) was added MeOH (1 mL) and BF_3OOEt_2 (1.4 mL, 11.3 mmol). The resulting mixture was cooled to 0°C and $Pb(OAc)_4$ (0.54 g, 1.21 mmol) was added. The reaction was warmed to 25°C with stirring for 45 min and then $NaHCO_3$ solution (sat., 30 ml) was added, and the mixture was stirred for 1 h. It was poured into a separatory funnel containing water (50 mL) and extracted with ether (3x40 mL). The ethereal solution was washed with brine (50 mL) and then dried over K_2CO_3. The crude product obtained by removal of solvent was dissolved in MeOH (25 mL) and K_2CO_3 solution (sat., 8 mL) was added. The reaction was stirred for 5 h and then the reaction mixture was poured into a separatory funnel containing 50 mL of water. It was then extracted with CH_2Cl_2 (3x30 mL). The combined extracts were dried over K_2CO_3 and the crude material obtained was purified by chromatography to give the product (160 mg) along with 21-methoxy by-product (40 mg). The product was further purified by recrystallization

from 10% acetone in hexane to give 88 mg of the pure product (28%) as a white solid, m.p. 140-142°C.

EXAMPLE 26

a. 3α-Hydroxy-3β-methyl-5β-19-nor-pregn-(Z)17(20)-ene:

To a solution of 5β-19-nor-pregn-17(z)-en-3-one (600 mg, 2.1 mmol) in THF (20 mL) at -78°C was added methylmagnesium bromide (3 M in ether, 1 mL, 3 mmol). The reaction was warmed to 25°C. Ammonium chloride solution (50 mL) was added and the mixture was extracted with ether (3x30 mL). The product (577 mg, 91%) purified by chromatography (10% acetone in hexane) was obtained as a white waxy solid.

b. 3α-Hydroxy-3β-methyl-5β-19-nor-pregnan-20-one:

To a solution of 3α-hydroxy-3β-methyl-5β-19-nor-17(z)-pregn-17(20)-ene (0.57 g, 1.9 mmol) in THF (30 mL) was added BH_3•THF solution (1 M in THF, 3.8 mL, 3.8 mmol) at 25°C. The reaction was completed in 30 min and NaOH solution (20%, 8 mL) was added very cautiously followed by a addition of hydrogen peroxide solution (30%, 8 mL). The mixture was stirred for 20 min and NH_4Cl solution was then added. The mixture was extracted with CH_2Cl_2 (3x30 mL) and the combined extracts were dried over K_2CO_3. Removal of the solvent left the crude product (760 mg) which was dissolved in CH_2Cl_2 (40 mL). 4-Methylmorpholine *N*-oxide (0.6 g, 4.75 mmol) and freshly ground molecular sieves (4Å, 5 g) were added and the resulting mixture was stirred for 20 min. The catalyst tetrabutylammonium perruthenate (100 mg) was added. The oxidation was complete within 40 min and the reaction mixture was filtered through a pad of Florisil which was rinsed with a 2:1 mixture of ether and CH_2Cl_2 (60 mL). The crude material (0.62 mg) obtained by the removal of the solvent was purified by chromatography (20% EtOAc in hexane) to give the product (53 mg, 9%) as a white solid.

EXAMPLE 27

3β-Ethynyl-3α,20α-dihydroxy-5β-pregnane and 3β-ethynyl-3α,20β-dihydroxy-5β-pregnane

To a solution of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one (0.31 g, 0.91 mmol) in methanol (20 mL) was added sodium borohydride (200 mg, 5.3 mmol) and it was stirred at 25°C for 1 h. Ammonium chloride (50 mL) solution was then added and the mixture was extracted with CH_2Cl_2 (3x30 mL). A chromatography (EtOAc:Hex=3:7) gave 3β-ethynyl-3α,20β-dihydroxy-5β-pregnane (200 mg, 65%) as major product: m.p., 221-223°C. The minor product, 3β-Ethynyl-3α,20α-dihydroxy-5β-pregnane was further purified by another chromatography (25-30% ethyl acetate in hexane, 16 mg, 5%): m.p., 187-188°C.

EXAMPLE 28

a. 3α,21-dihydroxy-3β-ethynyl-5β-pregnan-20-one

To a solution of 3α,21-dihydroxy-3β-ethynyl-5β-pregnan-20-one 21-acetate (725 mg, 1.81 mmol) in 45 mL of methanol at 0°C was added an aqueous 10% K_2CO_3 solution (3.75 mL). After stirring for 30 m at rt, the mixture was recooled to 0°C and a 2N aqueous HOAc solution (1.8 mL) was added. The reaction was added to an EtOAc/water mixture. The aqueous layer was extracted twice with EtOAc and the pooled organic layers were extracted with a brine solution, dried (Na_2SO_4) and concentrated. Purification by flash chromatography (20 cm of silica gel in a 4 cm dia. column, eluted with 2 liters of 20% acetone/hexane) gave 582 mg (90%) of the diol as a white solid, mp 155.5-157°C.

b. 3β-Ethynyl-3α-hydroxy-21-methoxy-5β-pregan-20-one

A solution of 3β-ethynyl-3α-hydroxy-5β-pregan-20-one (503 mg, 1.47 mmol) in methanol (4 mL) and toluene (17 mL) was cooled in an ice/water bath and neat BF_3-Et_2O (2.9 mL, 3.35 g, 23.6 mmol) was added followed by 1.0 g (2.25 mmol) of solid lead tetraacetate. The mixture was allowed to

warm to r.t. and then stirred for 80 min. The reaction was then added to 25 mL of an ice-cold sat. $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (2 x 10 mL) and the combined organic layers were extracted with a sat. NaCl solution, dried (Na_2SO_4) and concentrated. Two flash columns (silica gel, eluted with 2.5% acetone/CH_2Cl_2) afforded 148 mg (27%) of the title compound as a white solid, mp 198.5-199.5°C.

EXAMPLE 29

Preparation of (3-*R*)-20,20-ethylenedioxy-5α-pregnane-spiro-2'-oxirane

A mixture of trimethylsulfoxonium iodide (Aldrich; 8.79 g, 39.9 mmol) and solid potassium *tert*-butoxide (Aldrich, 95%; 4.3 g; 36.6 mmol) in 125 mL of dry THF was heated at reflux for 1.5 h. The reaction was allowed to cool to r.t. and 20,20-ethylenedioxy-5α-pregnan-3-one (12.0 g, 33.3 mmol) was added as a solid in one portion. The resulting mixture was stirred at r.t. for 20h. and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and extracted with water. The aqueous layer was back extracted with CH_2Cl_2 and the combined organic layers were washed with a saturated NaCl solution, dried ($MgSO_4$) and concentrated. The white solid obtained (12.0 g, 96%, mp 160-165°C) was recrystallized from 60/40 acetone/methanol.

EXAMPLE 30

3α,20-dihydroxy-3β,20-dimethyl-5α-pregnane

5α-Pregnan-3,20-dione (1.5 g, 4.7 mmol) was dissolved in 250 mL of dry benzene and 15 mL of 3M methylmagnesium bromide was added. The reaction mixture was stirred at room temperature for 30 min. The excess methyl magnesium bromide was decomposed by adding 100 mL of sulfuric acid dropwise at 0°C. The product solution was extracted with 250 mL of diethyl ether. The organic extract was combined, dried over magnesium sulfate, and evaporated to give a 60:40

mixture of 3β-methyl and 3α-methyl isomers. The product mixture was separated by flash chromatography on silica gel using a 70:30 (hexane:ethyl acetate and 1% methanol). elution to give 0.476 g (24% yield) of the desired product.

EXAMPLE 31

3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one

A solution of 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one, 21-acetate (1.36g, 3.06mmol) in MeOH (75mL) was cooled to 0°C. A solution of K_2CO_3 (10% aqueous, 6.45mL, 4.67mmol) was then added dropwise. After stirring for 1.5 hours at 0°C, a solution of acetic acid (2N aqueous, 2.5mL, 5.0mmol) was added dropwise and the mixture was allowed to warm to room temp. EtOAc, CH_2Cl_2 and water (100mL each) were added and thoroughly mixed. The organic phase was isolated, washed with aqueous $NaHCO_3$ and NaCl solutions, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash column chromatography (hexane/EtOAc 3:1) to yield a white solid (973mg, 79%). mp. 148-150°C.

EXAMPLE 32

a. 3α-Hydroxy-5α-21-ethyl-pregn-17(z)-ene

To a Wittig reagent prepared by stirring the mixture of *n*-butyltriphenylphosphonium bromide (3.9 g, 9.66 mmol) and potassium *t*-butoxide (1.1 g, 9.66 mmol) in THF (10 mL) for 30 min was added 3α-hydroxy-5α-androstan-17-one (0.7 g, 2.4 mmol) and the mixture was heated to reflux for 16 h. The reaction was then quenched with ammonium chloride solution and extracted with CH_2Cl_2. The pure product (230 mg, 29%) was obtained by chromatography (10% acetone in hexane).

b. 3α-t-Butyldimethylsiloxy-5α-21-ethyl-pregn-17(z)-ene

The mixture of *t*-butyldimethylsilylchloride (0.32 g, 2.1 mmol) imidazole (0.24 g, 3.5 mmol) and 3α-hydroxy-5α-

21-ethyl-pregn-17(z)-ene (0.23 g 0.7 mmol) was stirred for 12 h at 25°C. Ammonium chloride solution was then added and extraction was done with ether (3x30 mL). The obtained extract was washed with brine (3x30 mL). The pure product (0.27 g, 87%) was then obtained by chromatography (Hexane).

c. <u>3α,20α-Dihydroxy-5α-21-ethylpregnane</u>

To a solution of 3α-t-butyldimethylsiloxy-5α-21-ethyl-pregn-17(Z)-ene (0.27 g, 0.6 mmol) in THF (20 mL) was added BH_3•THF (1M in THF, 1.2 mL) at 25°C. Then sodium hydroxide (20%, 5 mL) was added cautiously after the reaction was stirred for 1 h. Then hydrogen peroxide solution (30%, 5 mL) was added and the mixture was stirred for another hour. Ammonium chloride solution was then added and it was extracted with CH_2Cl_2. The 20α-hydroxy-3α-silyl ether compound was obtained by chromatography (5% acetone in hexane). 60 mg of this compound was desilylated using hydrofluoric acid in acetonitrile. Chromatography (20-30% ethyl acetate in hexane) gave 11 mg of the pure title compound (m.p., 229-232°C, Rf=0.28, EtOAc:Hex=3:7).

EXAMPLE 33

<u>3β-Fluoromethyl-3α-hydroxy-5α-pregnan-20-one</u>

A mixture of *n*-Bu_4NF•xH_2O (7.873 g) and benzene (50 mL) was refluxed with a Dean-Stark trap overnight. The mixture, which was not a clear solution, was then concentrated (normal pressure) to ~10 mL and allowed to cool to room temperature. A solution of 3(*R*)-5α-pregnan-3-spiro-2'oxirane-20-one ethyleneketal (2.55 g, 6.81 mmol) in dry benzene (15 mL + 5 mL for the rinse) was added, using a double-ended needle, to the above concentrated solution. The resulting solution was concentrated, using a short-path distillation apparatus, to ~10 mL and refluxed for 15 min. Since it was very difficult to spot the concentrated reaction solution on the TLC plate, dry benzene (5 mL) was added. TLC (100:1 CH_2Cl_2/acetone or 3:1 hexane/acetone)

showed some unreacted starting material, besides two new less-polar spots. The reaction mixture was again concentrated and then refluxed for 30 min; TLC (after diluting the mixture with benzene) now showed almost complete consumption of the starting epoxide. As before, the mixture was concentrated and refluxed for a while. <u>It must be noted here that this reaction takes place only when the mixture is highly concentrated.</u> After the mixture had come to room temperature (giving a light-yellow solid), ether and water were added. Since all the solid did not dissolve, CH_2Cl_2 was added. The aqueous layer was back extracted with CH_2Cl_2. The combined organic extracts were washed with water (x2), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to provide a white solid (3.33 g) whose ^{1}H NMR showed it to be a 4:1 mixture of 3β-fluoromethyl-3α-hydroxy-5α-pregnan-20-one ethyleneketal and 3α-fluoro-3β-hydroxymethyl-5α-pregnan-20-one ethylene ketal.

In order to hydrolyze the ketal, acetone (100 mL), water (5 mL), and p-TsOH•H_2O (143 mg, 0.752 mmol) were added to the above solid. The pH was adjusted by adding in HCl until slightly acidic. The mixture was heated with a heat gun for a while in order to get a clear solution, which was stirred at room temperature for 2 h. The mixture became turbid, so CH_2Cl_2 was added to obtain a clear solution. TLC indicated completion of the reaction. The solvent was removed under reduced pressure, giving a white solid to which were added CH_2Cl_2 and water. The aqueous layer was back-extracted with CH_2Cl_2. The combined organics were washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to furnish a white crystalline solid (2.5 g). Flash column chromatography of mecrude product with CH_2Cl_2 as eluent provided the desired 3β-fluoromethyl-3α-hydroxy-5α-pregnan-20-one (1.41 g, 59%). mp 201-3°C.

EXAMPLE 34

3α-hydroxy-3β-methyl-5α-pregn-17(20)-ene

To a solution of 5α-pregn-17(20)-en-3-one (207 mg, 0.689 mmol) in 8 mL of dry THF at -30°C (At -78°C, not all of the steroid dissolved) was added a 3M solution of MeMgBr in ether (Aldrich; 0.34 mL, 1.02 mmol) dropwise via syringe. After stirring at -30°C for 4.75 h, the reaction was added to a sat. NH_4Cl solution/ether mixture. The aqueous layer was extracted with ether (3x30 mL) and the organic layers were combined and washed with a brine solution, dried ($MgSO_4$) and concentrated at reduced pressure. The crude material was dissolved in CH_2Cl_2 and added to 32 cm of flash silica gel in a 2 cm column. The column was eluted with 100% CH2Cl2 until unreacted ketone (8.7 mg, 4%) had eluted. Elution with 100mL each of 2.5 and 5% acetone/CH2Cl2 gave 38 mg (18%) of 3α-hydroxy-3β-methyl-5α-pregn-17(20)-ene (R_f 0.35, 100% CH_2Cl_2) and 3β-hydroxy-3α-methyl-5α-pregn-17(20)-ene (R_f 0.16, 100% CH_2Cl_2).

EXAMPLE 35

3α-hydroxy-17Z- and 17E-methoxymethylene-5β-androstane

Solid 3α-hydroxy-5β-androstan-17-one (504 mg, 1.74 mmol) was added in portions to the ylide prepared from methoxymethyltriphenylphosphonium chloride (Aldrich; 1.484 g, 4.33 mmol) and potassium *tert*-butoxide (479 mg, 4.27 mmol). This mixture was refluxed for 7h and then cooled to 0°C and added to an ice-cold sat. NH_4Cl solution/ether mixture. The aqueous layer was extracted twice with ether and the combined organic layers were washed with a sat. NaCl solution, dried ($MgSO_4$) and concentrated. The crude alkene was dissolved in CH_2Cl_2 and added to 13 cm of flash silica in a 3.5 cm dia. column. The column was eluted with 400 mL of 5% acetone/CH_2Cl_2 and 200 mL of 7.5% acetone/CH_2Cl_2. The desired alkene was isolated as a 1:1 mixture of *Z*- and *E*-isomers (158 mg, 28%) along with recovered 17-one (220 mg, 44%). The mixture of alkene

isomers was resubjected to flash chromatography (15 cm of silica in a 3.5 cm dia. column, eluted with 500 mL of 4% acetone/toluene and 200 mL each of 5,6, and 7% acetone/toluene) without good separation. Preparative HPLC (21.4 mm id x 25 cm Microsorb 5m column; solvent system 2.5% acetone/CH_2Cl_2, 10 mL/min; RI detection) allowed the separation of *Z*-isomer, mp 150-152°C, (more polar) and *E*-isomer, mp 151-153°C (less polar).

EXAMPLE 36

3α,20β-Dihydroxy-3β-methyl-5α-pregnane and 3α,20α-Dihydroxy-3β-methyl-5α-pregnane

Reference: House, H. O.; Muller, H. C.; Pitt, C. G.; Wickam, P. P. *J. Org. Chem.* 1963, *28*, 2407.

To a mixture of freshly cut sodium (250 mg, 10.87 mmol) and boiling toluene (5 mL) were added dropwise over a period of 30 min a solution of 3α-hydroxy-3β-methyl-5α-pregnan-20-one (340 mg, 1.022 mmol) in isopropyl alcohol (3 mL, 39.18 mmol) and toluene (4 mL + 2 mL for the rinse). The resulting mixture was refluxed for 2.5 h at which point TLC (3:1 hexane/acetone) indicated completion of the reaction and displayed two new spots that had lower R_f's than the starting ketone and had approximately same intensity. The reaction solution was allowed to come to room temperature and then was cooled in an ice-bath. 1N HCl (10 mL) was added dropwise. After diluting the mixture with CH_2Cl_2 (~50 mL), the aqueous layer was separated and back-extracted with CH_2Cl_2 (x2). The combined organics were washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white solid (354 mg). Purification of these two products was accomplished by flash chromatography employing 15:1 and 10:1 hexane/acetone as eluant.

Evaporation of the early fractions provided 3α,20β-dihydroxy-3β-methyl-5α-pregnane (80 mg) as a white solid.

^1H NMR and capillary GC were employed to determine the purity.

Further elution provided a 9:1 mixture (on the basis of GC) of 3α,20α-dihydroxy-3β-methyl-5α-pregnane and 3α,20β-dihydroxy-3β-methyl-5α-pregnane (total 248 mg). This mixture was subjected to recrystallization from hot ethanol (~10 mL); No crystals were obtained. Attempted next was hot 5:1 hexane/acetone (~50 mL), which led to white crystals (110 mg). According to GC, these crystals had a purity of only 96%. Finally, pure 3α,20α-dihydroxy-3β-methyl-5α-pregnane (80 mg) could be obtained by another recrystallization from hot ethanol.

EXAMPLE 37

3α-Hydroxy-3β-iodomethyl-5α-pregnan-20-one

To a solution of 3[R]-spiro-2'-oxirane-5α-pregnan-20-one (40 mg, 0.12 mmol) in acetone (3 mL) was added NaI (39 mg, 0.26). The resulting solution was stirred at room temperature for ~4 h. TLC (3:1 hexane/acetone) indicated consumption of only a small amount of the starting material, with appearance of a new, slow-moving spot; the relative intensities of these two spots did not change with time, suggesting presence of an equilibrium between the starting material and the iodomethyl product (as alkoxide). Subsequently acetic acid (~0.02 mL) was added to the above reaction solution. The mixture was stirred at room temperature overnight. TLC now showed complete disappearance of the starting epoxide. The reaction mixture contained some white crystals (sodium acetate). The solvent was removed under reduced pressure, and then ether and water were added. The aqueous phase was separated and back-extracted with ether. The combined ether solutions were successively washed with water and brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give 3α-hydroxy-3β-iodomethyl-5α-pregnan-20-one as white solid (51 mg, 92%).

EXAMPLE 38

a. 3,3-Ethylenedioxy-5β-pregn-17(20)-ene

5β-3-Ethylenedioxy-androstan-17-one (6 g) was added to Wittig reagent produced from ethyltriphenylphosphonium bromide (15 g) and potassium *t*-butoxide (4.5 g) in THF (15 mL). The reaction was refluxed for 2 h and cooled to 25°C. Then methylene chloride (80 mL) and a solution of ammonium chloride (60 mL) was added and the organic layer was separated by separatory funnel. The aqueous layer was extracted with methylene chloride (2x50 mL). The organic solution was dried over potassium carbonate and the solvent removed in vacuo. Most of the phosphoroxide was removed by washing with hexane. The obtained product (6 g) was dissolved in acetone (100 mL), and hydrochloric acid (2 N, 10 mL) added. The crude product (5.5 g) obtained by a basic work-up followed by methylene chloride extraction was purified by chromatography to produce 4.5 g of product (83%). The pure stereoisomer was obtained by repeated recrystalization from hexane.

b. 3α-Hydroxy-3β-trifluoromethyl-5β-pregn-(Z)17(20)-ene

To a solution of 5β-pregn-(Z)-17(20)-en-3-one (950 mg, 3.17 mmol) in THF (15 mL) was added trifluoromethyl-trimethylsilane (0.5 M solution in THF, 9.5 mL) at 0°C. The solution turned to brown gradually and the reaction was finished in 30 min. Water (30 mL) was added and organic layer collected. The aqueous layer was extracted with ether (3x50 mL) and organic solution was dried over potassium carbonate. The pure product (680 mg, 58%) was isolated by column chromatography using ethyl acetate and hexane (1:9) as eluent.

EXAMPLE 39

a. 3α,21-Dihydroxy-3β-ethynyl-5β-pregnan-20-one 21-acetate

A suspension of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one (1.00 g, 2.92 mmol) in 35 mL of toluene was treated with 2 mL of methanol. The resulting solution was cooled in an ice/water bath and neat BF_3-Et_2O (Aldrich; 5.8 mL, 5.02 g, 35.4 mmol) was added. Solid lead tetraacetate (Aldrich; 1.96 g, 4.42 mmol) was added in a few portions. A light purple solution formed initially and became light brown as stirring continued at 0°C. The mixture was stirred at r.t. for 3 h. and then recooled to 0°C. The cold reaction was added to a mixture of 52 mL of a sat. $NaHCO_3$ solution, water and crushed ice. The resulting mixture was extracted with EtOAc (2 x 75 mL). The combined organic layers were extracted with a sat. NaCl solution, dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (25 cm of flash silica gel in a 5 cm dia. column eluted with 3 liters of 20% acetone/hexane) affording 749 mg (64%) of the acetate, mp 196-198°C.

b. 3α,21-Dihydroxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate

To a stirred solution of 21-bromo-3β-ethynyl-3α-hydroxy-5β-pregnan-20-one (1.5g, 3.56 mmol) in 50 mL of acetone was added succinic acid (2.1g, 17.8 mmol) and triethylamine (3mL, 21.4 mmol). The reaction was heated to reflux for 3.5 h. then cooled to r.t. before partitioning between ethyl acetate and dilute aq. HCl. The organic layer was washed with sat. NaCl, dried with Na_2SO_4 and concen-trated *in vacuo* to give a crude solid. The solid was triturated with dichloromethane to remove succinic acid as a solid, and the liquid was concentrated *in vacuo* to give a foam (1.36g). Flash chromatography on 6 in. of silica gel in a 3cm column collecting 50 mL fractions and

eluting with 1:3 -> 3:1 ethyl acetate : hexane with a gradual adjustment of the gradient to give 830mg (51%) of the title compound.

c. Sodium 3α,21-dihydroxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate

The hemisuccinate, 633 mg, mp 62-68 °C, was dissolved in methanol and an aqueous solution of 116 mg (0.253 mmol) of $NaHCO_3$ was added. After stirring for 3.5 h, the solvent was removed under reduced pressure and the residue was triturated with an ether/hexane mixture. The light yellow solid obtained, weight 616 mg, was soluble in water at > 20 mg/mL.

EXAMPLE 40

3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one, 21-acetate

Under an anhydrous argon atmosphere, 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one (1.94g, 5.02mmol) was dissolved in toluene (86mL) and MeOH (5.2mL). Boron trifluoride etherate (10.4mL, 84.3mmol) was added via syringe. Lead tetraacetate (2.89g, 6.51mmol) was then added. The mixture was stirred for 70min, poured into water and extracted three times with CH_2Cl_2. The organic phases were combined, washed with aqueous $NaHCO_3$ and NaCl solutions, dried over $MgSO_4$ and evaporated *in vacuo* to give a pale yellow solid (2.18g). This solid was purified by flash column chromatography (CH_2Cl_2/EtOAc 150:1 and hexane/EtOAc 4:1) to give a white solid (1.54g; 69%). mp. 167-168.5°C.

EXAMPLE 41

3β-Chloromethyl-3α-hydroxy-5α-pregnan-20-one

A mixture of 3[R]-spiro-2'-oxirane-5α-pregnan-20-one (46 mg, 0.14 mmol), tetramethylammonium chloride (25 mg, 0.23 mmol) and dry DMF (Aldich; 5 mL) was heated at ~130°C.

A clear solution was obtained after some time. TLC (7:1 hexane/acetone) showed, besides the starting material, a new spot that moved slower than the starting epoxide. There was no further progress in the reaction after 2 h. More tetramethylammonium chloride (~25 mg) was added to the reaction mixture. The resulting mixture was stirred at 130 - 150°C for 1 h. Not all the solid dissolved. There was no change in TLC. Presumably there is an equilibrium between the chloromethyl product (as alkoxide) and the starting epoxide. Acetic acid (0.1 mL, 1.75 mmol) was then added to the reaction mixture while the mixture was still very hot. All the solid particles that were present in the mixture now dissolved, and a clear solution resulted. This solution was heated at ~100°C for 2 h at which point TLC indicated completion of the reaction, displaying only one spot. The solvent was removed by distillation under vacuum. To the resulting solid were added CH_2Cl_2 and water. The aqueous layer was back-extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a solid (46 mg). ^{1}H NMR of this solid showed only the desired regioisomer (i.e., 3β-chlororomethyl-3α-hydroxy-5α-pregnan-20-one). Finally, flash chromatography employing 9:1 hexane/acetone as eluant provided the title compound (35 mg, 68%) as a white solid. m.p.209-10°C (dec.).

EXAMPLE 42

a. 3β-(3'-Bromo-1-propynyl)-3α-hydroxy-5β-pregnan-20-one

A solution of propargyl bromide (80% soln. in toluene, 0.4 mL, 3.6 mmol) in dry THF (7 mL) was treated with n-BuLi (2.5M in THF, 3 mmol, 1.2 mL) at -78°. After stirring the mixture at this temp. for 10 min. a solution of 5β-pregnan-3,20-dione, 20-ketal (360 mg, 1 mmol) was added and the mixture was stirred at -78°C for 2 hr. It was then quenched with sat. NH_4Cl solution (1 mL). The solvent was removed and the residue was then dissolved in acetone (40

mL). After adding 2N HCl (10 mL) the solution was stirred at r.t. for 3.5 h. Sat. $NaHCO_3$ soln. was added to neutralize the acid. The solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (360 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with hexane:acetone mixture (90:10) gave 3α-(3'-bromo-1-propynyl)-3β-hydroxy-5β-pregnan-20-one (50 mg) as a first fraction. Further elution with the same solvent mixture yielded 3β-(3'-bromo-1-propynyl)-3α-hydroxy-5β-pregnan-20-one (181 mg).

EXAMPLE 43

3β-Bromomethyl-3α-hydroxy-5α-pregnan-20-one

A mixture of 3[R]-spiro-2'-oxirane-5α-pregnan-20-one (362 mg, 1.10 mmol), tetramethylammonium bromide (322 mg, 2.16 mmol), acetic acid (1.0 mL, 17.5 mmol), and dry DMF (Aldich; 30 mL) was heated at ~130°C. There was still some undissolved tetramethylammonium bromide present in the mixture, which was stirred at 110 - 120°C. TLC (3:1 hexane/acetone) after heating for 30 min at 110 -120°C showed, besides the starting material, a new, more-polar spot. There were also two other very light spots that moved slower than the major product spot; the intensity of these two very light spots increased with time. After 3 h at 110 -120°C, TLC displayed three spots of almost equal intensity, besides the spot corresponding to the unreacted starting material whose amount did not change with time. The solvent was removed by distillation under vacuum. To the resulting solid were added CH_2Cl_2, water, and brine (to prevent the emulsion formation). The organic layer was washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a solid (380 mg). ^1H NMR of this solid indicated presence of three products,

namely, 3β-bromomethyl-3α-hydroxy-5α-pregnan-20-one, 3-hydroxymethyl-5α-pregn-2-en-20-one, and 3β-(acetoxymethyl)-3α-hydroxy-5α-pregnan-20-one and the unreacted starting material. Finally, flash chromatography employing 9:1 hexane/acetone as eluent provided pure 3β-bromomethyl-3α-hydroxy-5α-pregnan-20-one (98 mg, 22%) as a white solid. m.p. 196- 97°C (dec.).

EXAMPLE 44

a. 3α-Hydroxy-19-nor-5α-17-methoxymethleneandrostane

To a solution of 19-nor-5α-17-methoxymethleneandrostan-3-one (200 mg, 0.65 mmol) in THF (20 mL) was added K-selectride (1 M in THF) by a syringe at -78°C and the reaction was stirred at this temperature for 4.5 h. Then mixture was poured into a separatory funnel containing ammonium chloride solution (saturated, 20 mL) and methylene chloride (40 mL). The aqueous layer was extracted with methylene chloride (2x15 mL). The organic solution was dried over potassium carbonate and the solvent removed in vacuo. A column chromatography (4% acetone, 4% methylene chloride in hexane) resulted 130 mg product (64%).

b. 3α-Hydroxy-19-nor-5α-androstan-17β-carboxaldehyde

To a solution of 3α-hydroxy-19-nor-5α-17-methoxymethleneandrostane (130 mg, 0.43 mmol) in THF (10 mL) was added a solution of hydrochloric acid (1N, 1 mL) at 25°C and the reaction was stirred for 6 h. Then sodium bicarbonate solution ($NaHCO_3$, saturated, 20 mL) and methylene chloride (30 mL) were added. The organic layer was separated and aqueous layer extracted with methylene chloride (2x20 mL). The organic solution was dried over potassium carbonate and the solvent removed in vacuo. The pure product (80 mg, 65%) was isolated by column chromatography using acetone and hexane (1:4) as eluent.

EXAMPLE 45

3α,21-Dihydroxy-3β-methyl-5α-pregnan-20-one 21-Disodium phosphate.

To a solution of 21-bromo-3α-hydroxy-3β-methyl-5α-pregnan-20-one(1.0 g, 2.43 mmol) in 10 mL THF at RT was added dibenzyl hydrogen phosphate(2.1 g, 7.3 mmol) and triethylamine (1.085 mL, 7.53 mmol) with stirring. The reaction was then heated to reflux for 4.5 h and then cooled to RT. Dichloromethane(25 mL) was then added and the solution transferred to a separatory funnel, washed with 1N HCl, sat. aq. $NaHCO_3$, dried with $MgSO_4$ and concentrated in vacuo to give the dibenzylphosphate as a crude oil (1.205 g). The dibenzylphosphate (790 mg, 1.3 mmol) was dissolved in 2 : 1 EtOH : THF (30 mL) with a few drops of sulfuric acid, charged with 5% Pd/C (180 mg, 20% by weight) and subjected to 50 psi of H_2 at rt until the reaction was complete by TLC. The catalyst was removed by filtration and the filtrate was concentrated. The residue was dissolved in 4 : 1 MeOH : water (10 mL) and titrated to pH 11 with 1M NaOH. (The solution was cloudy) The solution was treated with acetone until a readily filter-able solid precipitated after which the mixture was cooled to 0°C and filtered to isolate a crude solid 260 mg. The solid was dissolved in 20 mL water forming a cloudy solution that was filtered and then concentrated to give 220 mg of the title compound as a white solid.

EXAMPLE 46

a. 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate

Succinic acid (20 g, 170 mmol) and triethylamine (19 mL, 140 mmol) were added to a solution of crude 21-bromo-3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one (5.36 g; ≤ 10.9 mmol) in acetone (200 mL). The mixture was refluxed for 1.75 hr. The residue was poured into ice water (350 mL). More water (100 mL) was then added. The mixture was stirred and then extracted three times with CH_2Cl_2 (200 mL, 200 mL and 100 mL). The combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo* to give a semi-solid residue (5.71 g) which was purified by flash column chromatography (CH_2Cl_2/MeOH; gradient 90:1 to 20:1) to give a white solid (3.63 g, 66% over 2 steps). mp: dec. (over broad range).

b. 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one, 21-hemisuccinate, sodium salt

3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one, 21-hemisuccinate (859mg, 1.71mmol) was dissolved in MeOH (35mL). $NaHCO_3$ (143mg, 1.70mmol) in water (10mL) was then added dropwise. More MeOH (10mL) was added. After stirring for 2.5 hours, the solvent was evaporated. Toluene was added and evaporated *in vacuo*. The residue was treated with ether/hexane and then with hexane to give a white solid. MeOH was added and removed. Heptane was added and removed *in vacuo*. The residue was treated with hexane and dried *in vacuo* to give a white solid (878mg, 98%).

EXAMPLE 47

a. 21-(N,N-dimethylamino)methyl-3α-hydroxy-3β-methyl-5α-pregnan-20-one

A solution of 3α-hydroxy-3β-methyl-5α-pregnan-20-one (3.32 g, 10 mmol) and N,N-dimethylmethyleneammonium chloride (1.6 g, 17 mmol) in acetonitrile (15 mL) was refluxed

for 2.5 hr. After cooling the solvent was removed and the residue was made basic by adding sat. $NaHCO_3$ soln. The mixture was then extracted with CH_2Cl_2, washed with brine, dried over anhyd. $MgSO_4$, and evaporated to yield the crude product. This product was crystallized from acetone to give 21-(N,N-dimethylamino)methyl-3α-hydroxy-3β-methyl-5α-pregnan-20-one as colorless rods (550 mg).

The mother liquor was evaporated to dryness and the residue was chromatographed over silica gel. Elution with hexane:acetone (2:3, 1:4) afforded 21-(N,N-dimethylamino) methyl-3α-hydroxy-3β-methyl-21-methylene-5α-pregnan-20-one as a colorless solid (200 mg). Further elution with acetone yielded more 21-(N,N-dimethylamino)methyl-3α-hydroxy-3β-methyl-5α-pregnan-20-one as colorless solid (1 g).

b. <u>3α-hydroxy-3β-methyl-21-methylene-5α-pregnan-20-one</u>

A mixture of 21-(N,N-dimethylamino)methyl-3α-hydroxy-3β-methyl-5α-pregnan-20-one (500 mg) and MeI (4 mL) was stirred at r.t. for 1.5 hr. 4 mL of CH_2Cl_2 was added and the stirring was continued for another 15 hr. MeI and CH_2Cl_2 were removed and the residue was taken up in CH_2Cl_2 (20 mL) and was treated with sat. NaHCO3 solution (15 mL). After stirring at r.t. for 2.5 hr the organic layer was separated, washed with brine, dried over anhyd. $MgSO_4$, and evaporated. The residue (500 mg) was chromatographed over silica gel. Elution with CH_2Cl_2:acetone (95:5) gave 3α-hydroxy-3β-methyl-21-methylene-5α-pregnan-20-one (300 mg).

c. <u>3α-hydroxy-3β,21-dimethyl-5α-pregnan-20-one</u>

A solution of 3α-hydroxy-3β-methyl-21-methylene-5α-pregnan-20-one (250 mg) in EtOAc (30 mL) was hydrogenated (2.5 atm of H_2) in the presence of Pd/C (27 mg, 5%) at r.t. for 1 hr. After filtration and then removal of the solvent gave the title compound as colorless solid (198 mg); mp 190-192°C.

EXAMPLE 48

a. 21-(N,N-dimethylamino)methyl-3α-hydroxy-5β-pregnan-20-one

A solution of 3α-hydroxy-5β-pregnan-20-one (318 mg, 1 mmol) and N,N-dimethylmethyleneammonium chloride (100 mg, 1.1 mmol) in acetonitrile (3 mL) was refluxed for 2 hr. After cooling, the solvent was removed and the residue was made acidic by adding 5 mL of 2N HCl. The mixture was then extracted with EtOAc. The aqueous layer was separated and neutralized with 2N NaOH. The precipitated solid was extracted in CH_2Cl_2. The organic layer was separated, washed with brine, dried over anhyd. $MgSO_4$, and evaporated to yield the crude 21-(N,N-dimethylamino)methyl-3α-hydroxy-5β-pregnan-20-one as a colorless solid (200 mg), which was used as such for the next step.

b. <u>3α-hydroxy-21-methylene-5β-pregnan-20-one and 3α-hydroxy-21-methoxymethyl-5β-pregnan-20-one</u>

A mixture of 21-(N,N-dimethylamino)methyl-3α-hydroxy-5β-pregnan-20-one (550 mg), CH_2Cl_2 (2 mL), and MeI (4 mL) was stirred at r.t. for 1 hr. MeI and CH_2Cl_2 were removed and the residue was refluxed in MeOH for 72 hr. After cooling, the mixture was treated with ice-water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over anhyd. $MgSO_4$, and evaporated. The residue (500 mg) was chromatographed over silica gel. Elution with CH_2Cl_2:acetone (95:5) gave 3α-hydroxy-21-methylene-5β-pregnan-20-one (150 mg). Further elution with the same solvent afforded 3α-hydroxy-21-methoxymethyl-5β-pregnan-20-one as a colorless solid (120 mg).

EXAMPLE 49

a. <u>3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt</u>

3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-dibenzylphosphate (712 mg, 1.07 mmol) and palladium on activated carbon (5% Pd, 180 mg) in ethanol (20 mL) were

put under a 50 psi atmosphere of hydrogen (on the Parr apparatus) for 2 hours. The palladium on carbon was removed by filtration and the filtrate was evaporated giving an oil. This oil was dissolved in methanol/water 4:1 (10mL). 1.00 M NaOH (1.97 mL) was added dropwise to bring the pH to 11 (± 1). A small amount of solid was removed by filtration and most of the solvent from the filtrate was removed by evaporation. The remainder (mostly water) was removed by freeze-drying to give a white solid (494 mg, 88%). mp: 224° C dec. ^{1}H NMR available.

b. <u>3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-dibenzylphosphate</u>

3α-dihydroxy-3β-trifluoromethyl-21-bromo-5β-pregnan-20- one (1.20 g, 2.58 mmol), dibenzylphosphate (2.15 g, 7.74 mmol) and triethylamine (1.08 mL, 7.74 mmol) were stirred in THF (10 mL) and then heated to reflux for 3 hours. After cooling to room temp., EtOAc and 0.5 M HCl were added. The organic phase was isolated and washed with aqueous $NaHCO_3$ and NaCl, dried over $MgSO_4$ and evaporated *in vacuo* to give a brown oil. This oil was purified by flash column chromatography (hexane/acetone 4:1) to give a clear oil (712 mg, 42%).

c. <u>3α-hydroxy-3β-trifluoromethyl-21-bromo-5β-pregnan-20-one</u>

Hydrobromic acid (48%, 2 drops) was added to a stirred solution of 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one (4.20 g, 10.9 mmol) in methanol (100 mL). A solution of bromine (2.17 g, 13.6 mmol) in methanol (60 mL) was then added dropwise over a period of 10 min. After stirring for 30 min., solid $NaHCO_3$ was added (until all hydrobromic acid was neutralized) and the solvent was evaporated. The residue was partitioned between CH_2Cl_2 and water. The organic phase was isolated, washed with brine, dried over $MgSO_4$ and

evaporated *in vacuo* to give a yellow semi-solid residue (5.36 g) which was used without further purification.

d. <u>3α-hydroxy-3β-trifluoro-5β-pregnan-20-one</u>

(Reference: Krishnamurti, R.; Bellew, D.R.: Surya Prakash, G.K. <u>J. Org. Chem.</u> <u>56</u>:984 (1991))

To a solution of 5β-pregnan-3,20-dione 20-ethyleneketal (60 mg, 0.166 mmol) in dry THF (3 mL) was added 0.5 M $F_3CSi(CH_3)_3$ in THF (0.5 mL, 0.25 mmol). The resulting colorless solution was cooled to 0 °C., and η-Bu_4NFxH_2O (few crystals) was added. The cooling bath was removed, and the mixture was allowed to warm to r.t. Unlike the same reaction involving 5α-pregnan-3,20-dione 20-ethyleneketal, the above reaction mixture did not turn yellow and there was no gas generation. More 0.5 M $F_3CSi(CH_3)_3$ in THF (0.5 mL, 0.25 mmol) was added. The resulting mixture was stirred at room temperature for a few minutes. TLC (3:1 hexane/acetone) displayed a new spot with an R_f close to 1, but there was still some unreacted starting material present. Therefore, more 0.5 M $F_3CSi(CH_3)_3$ in THF (0.5 mL, 0.25 mmol) was added. The mixture was again stirred at room temperature for a while. No unreacted starting material was found. 1N HCl (~3 mL) was added, and the resulting two-phase mixture was stirred at room temperature overnight. The spot that was formed as a result of trifluoromethylation had now completely disappeared, and there were two new, less polar spots, the lower one being the major product. The mixture was then diluted with ether and water. The aqueous layer was separated and extracted with ether. The combined organice layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white crystalline (foamy) solid. The two epimers were separated by flash chromatography with 15:1 hexane:acetone. Evaporation of the early fractions gave the minor isomer. Further elution of the column gave 50 mg of the title compound.

EXAMPLE 50

a. 3β-Trifluoromethyl-3α-hydroxy-21-bromo-5β-19-nor-pregnan-20-one

To a flask containing a solution of 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one (0.65 g, 1.75 mmol) in methanol (10 mL) was added a solution of bromine (0.15 mL) in methanol (10 mL) dropwise in such a rate to maintain the brown color the bromine until this color was persistent. Then water (50 mL) was added the mixture was extracted with CH_2Cl_2 (3x40mL). The combined extracts were dried over K_2CO_3. Removal of the solvent resulted in the product as a foamy white solid (0.805 g).

b. 3β-Trifluoromethyl-3α-hydroxy-5β-19-nor-pregnan-20-one-21-dibenzylphosphate

To a solution of 3β-trifluoromethyl-3α-hydroxy-21-bromo-5β-19-nor-pregnan-20-one (0.805 g) in THF (10 mL) was added dibenzylphosphate (1.48 g, 5.32 mmol) and triethylamine (538 mg, 5.32 mmol) and the mixture was heated to reflux for 1.5 h. HCl (0.5 N, 40 mL) was added and it was extracted with CH_2Cl_2 (3x40 mL). The combined extracts were dried over Na_2SO_4. Removal of the solvent resulted in the crude material which was purified by chromatography to give 733 mg product (65%).

c. 3α-Hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one-21-phosphate, disodium salt

A mixture of 3β-trifluoromethyl-3α-hydroxy-5β-19-nor-pregnan-20-one-21-dibenzylphosphate (733 mg) and Pd/C (5%, 200 mg) in ethanol (20 mL) was attached to a Parr hydrogenator. The hydrogenolysis was done within 30 min. under about 50 psi and the resulting mixture was filtered through #5 filter paper to remove the catalyst. A white foamy solid (494 mg) was obtained after the solvent was removed. This solid was dissolved in methanol (10 mL) and a solution of $NaHCO_3$ (190 mg) in water (8 mL) was added at

0° C. The methanol was then removed *in vacuo* and the resulting aqueous solution was freeze-dried to give the product (530 mg, 92%).

EXAMPLE 51

3α-Hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one.

To a solution of 21-bromo-3α-hydroxy-5β-pregnan-20-one(2. 8 g, 7.05 mmol) and 4-mercaptopyridine(940 mg, 8.46 mmol) in 30 mL dry THF at 25° C was added triethylamine (1 mL) dropwise and the mixture heated to 60° C and stirred for 1.5 h. The solution was then partitioned between ethylacetate and 1:1 sat. $NaHCO_3$ and water. The organic layer was then washed with sat. NaCl, dried with $MgSO_4$ and concentrated *in vacuo* to a crude solid weighing 3.19g. Flash chromatography on 6 in. of silica gel in a 5.5 cm column collecting 50 mL fractions and eluting with 10%-15% acetone:CH_2Cl_2 resulted in 2.246g(75%) of the title compound as a yellow solid. MP 192-4° C.

EXAMPLE 52

a. 3β-Ethynyl-3α-hydroxy-5α-pregnan-20-one, 20-ketal

A 250 mL three neck flask equipped with a gas inlet, a thermometer and a condenser was charged with lithium acetylide-EDA complex (2.75 g, 90%, 27.5 mmol). Dry benzene (60 mL) was added and acetylene gas was bubbled through the mixture at a moderate rate. The mixture was then heated to 50-55° C in an oil bath and treated in parts with 5α-pregnan-3,20-dione, 20-ketal (9 g, 25 mmol). The stirring was continued at this temp. for 5 h. and then at r.t. for another 17 h. The resulting suspension was cooled to 10° C and was treated with sat. NaCl soln. (5 mL). The solvent was removed and water was added to the residue. The water insoluble product was collected by filtration, washed with water, and dried under vacuum. This crude product was then crystallized from EtOAc to yield 3α-ethynyl-3β-hydroxy-5α-pregnan-20-one, 20-ketal (3.35 g).

The mother liquor was evaporated to dryness and the residue was purified by column chromatography over silica gel. Elution with toluene:acetone mixture (92:8) gave the unreacted starting ketone (1.3 g) followed by 3β-ethynyl-3α-hydroxy-5α-pregnan-20-one, 20-ketal (1.3 g) as a second fraction. Further elution with the same solvent mixture yielded the more polar 3α-ethynyl-3β-hydroxy-5α-pregnan-20-one, 20-ketal (270 mg).

b. <u>3β-Ethynyl-3α-hydroxy-5α-pregnan-20-one</u>

3β-Ethynyl-3α-hydroxy-5α-pregnan-20-one, 20-ketal (550 mg) was dissolved in a mixture of acetone (20 mL) and 2N HCl (10 mL) and the mixture was stirred at r.t. for 15 h. The solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$ the solution was filtered and evaporated to yield the crude product (414 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (92:8) gave the title compound (280 mg), mp 175-177°C.

c. <u>3α-ethynyl-3β-nitrooxy-5α-pregnan-20-one</u>

A solution of 3α-ethynyl-3β-hydroxy-5α-pregnan-20-one, 20-ketal (2.15 g) in $CHCl_3$ (45 mL) was cooled to -20° C and treated with acetic anhydride (20 mL). Fuming nitric acid (4 mL) was then added and the mixture was stirred at this temp. for 45 min. After warming to -5° C, the yellow solution was poured into a mixture of 2N NaOH (70 mL) and water (150 mL) to yield the resultant solution of pH 3-4, which was then extracted with $CHCl_3$, washed with water, sat. $NaHCO_3$ solution, brine, dried ($MgSO_4$) and evaporated to yield the title compound as a viscous material (3 g), which was used as such for the next step.

d. 3β-Ethynyl-3α-hydroxy-5α-pregnan-20-one

Crude 3α-ehtynyl-3β-nitrooxy-5α-pregnan-20-one (3 g) from the above step was taken in a mixture of THF and water (30 mL, 1:1). $AgNO_3$ (516 mg) was added. After stirring at r.t. for 15 h. the solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$, the solution was filtered and evaporated to yield the crude product (2 g). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (93:7) gave 3β-ethynyl-3α-hydroxy-5α-pregnan-20-one (550 mg) as a first fraction.

EXAMPLE 53

a. Preparation of the lithium reagent from 1,2-dibromoethylene

A 250 mL three neck flask equipped with a N_2 gas bubbler, a thermometer, and a dropping funnel was charged with 1,2-dibromoethylene (cis/trans mixture, 98%, Aldrich, 2.1 mL, 26 mmol, mw = 186, d = 2.246). Dry THF (40 mL) was added and the solution was cooled to -78°C in a dry ice-acetone bath. n-BuLi (2.5M in THF, 20 mL, 50 mmol) was added dropwise over a period of 35 min. The mixture was stirred at this temperature for 40 min. and the resulting reagent was used immediately for the next step.

b. 3β-Ethynyl-3α-hydroxy-5α-pregnan-20-one 20-ketal

The above solution of the reagent in THF, which was maintained at -78°C, was treated dropwise with a solution of 5α-pregnan-3,20-dione, 20-ketal (4.68g, 13 mmol) in THF (50 mL) over a period of 1 hr. The temperature was maintained below -70°C during the addition. The stirring was continued at this temperature for 15 min. (100% conversion as detected by TLC). The cooling bath was removed and the resulting solution was quenched with 2N HCl (pH 6-7). The

solvent was removed and the residue was extracted with chloroform, the organic layer was separated, washed with water, and dried over anhydrous MgSO4. Removal of the solvent gave the title product (3.9 g) as an epimeric mixture with the corresponding 3β-hydroxy epimer (85:15).

EXAMPLE 54

3α-Hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one

To a solution of 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregn-17(20)-ene (2.6 g, 7.3 mmol) in THF (80 mL), was added diborane THF complex (1M solution in THF, 22 mL) dropwise at 25° C. The reaction was complete in 1 h. and then a solution of sodium hydroxide (20%, 50 mL) was added very slowly at 0° C followed by an addition of hydrogen peroxide (30%, 30 mL). Water was then added and the THF layer was separated by separatory funnel. The aqueous layer was extracted with methylene chloride (2x40 mL). The organic solution was dried over potassium carbonate and the solvent removed *in vacuo*. A quick column (hex:acetone=1:1) gave 1.8 g of product which was subjected to PCC oxidation (PCC, 2.1 g, 9.6 mmol; sodium acetate, 0.8 g, 9.6 mmol). The pure product (700 mg, 26%) was purified by column chromatography using ethyl acetate and hexane (15:85) as eluent; m.p. 151.5-153.0° C.

EXAMPLE 55

a. Synthesis of benzyl phenyl sulfoxide

To a solution of benzyl phenyl sulfide (Aldrich; 1.068 g, 5.33 mmol) in 25 mL of CH_2Cl_2 at -78° C was slowly added a solution of *m*-chloroperbenzoic acid (Aldrich, 50-60%; 760 mg, 2.64 mmol if 60%) in 10 mL of CH_2Cl_2. After warming to room temperature and stirring overnight, the solution was added to 20 mL of a saturated $NaHCO_3$ solution. The aqueous layer was separated and extracted with CH_2Cl_2 (2 x 10 mL). The pooled organic layers were then dried ($MgSO_4$) and concentrated. The residue was subjected to flash column

chromatography (silica gel, 10% acetone/hexane and 15% acetone/hexane) affording the sulfoxide (632 mg, 55%) as a white solid, mp 123-126° C.

b. <u>20,20-ethylenedioxy-3α-hydroxy-3β-[[2-(phenylsulfinyl)-2-phenyl]ethyl]-5α-pregnane</u>

A solution of diisopropylamine (Aldrich, freshly distilled from CaH_2; 0.5 mL, 361 mg, 3.57 mmol) in 2 mL of dry THF was cooled to -10° C and treated with a 1.6 M solution of *n*-BuLi in hexanes (Aldrich; 1.0 mL, 1.6 mmol) added dropwise via syringe. After 10 min., the reaction was cooled to -75° C and a solution of benzyl phenyl sulfoxide (347 mg, 1.60 mmol) in 5 mL of dry THF was added dropwise via syringe over 30 min. To the resulting deep yellow solution was added 297 mg (0.79 mmol) of solid 20,20-ethylenedioxy-3(*R*)-5α-pregnan-3-spiro-2'-oxirane. The reaction was allowed to warm to r.t. and then warmed to 50°C. After 5 h, the reaction was allowed to cool to r.t. and added to 30 mL of ice-cold water. The resulting mixture was extracted with EtOAc (3 x 20 mL). The combined EtOAc layers were back extracted with a sat. NaCl solution, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (silica gel, gradient from 100% CH_2Cl_2 to 20% acetone/CH_2Cl_2) affording the sulfoxide (405 mg, 86%) as a mixture of two diastereomers. This mixture was carried on to the elimination step.

c. <u>3α-hydroxy-3β-[2-(*E*)-phenylethenyl]-5α-pregnan-20-one</u>

A suspension of the sulfoxide (200 mg, 0.339 mmol) in 1.5 mL of *p*-isopropyltoluene containing 0.2 mL of 2,4,6-collidine (distilled from CaH_2) was heated in an oil bath at 135° C for 60 min. After cooling to r.t., the solution was allowed to stir overnight. A white precipitate formed and was isolated and washed with *p*-isopropyltoluene (3 x 1mL). By ^{1}H NMR and TLC, the precipitate (69 mg, 44%) was the desired20,20-ethylenedioxy-3α-hydroxy-2-(*E*)-phenylethenyl-

5α-pregnane. A solution of the ketal in acetone at 0° C was treated with a 1M HCl solution and stirred cold for one hour. The reaction was poured into an EtOAc/water mixture and the organic layer was washed with water and a sat. NaCl solution. After drying (Na_2SO_4), the solvent was removed *in vacuo* and the residue was purified by flash chromatography (silica gel, eluted with 1% acetone/CH_2Cl_2).

EXAMPLE 56

3α-hydroxy-3β-(2'-propenyl)-5β-pregnan-20-one

A solution of 5β-pregnan-3,20-dione, 20-ketal (180 mg, 0.5 mmol) in dry THF (20 mL) was treated with allyl magnesium bromide (2M in THF, 1 mmol, 0.5 mL) at -70° C. After stirring the mixture at this temp. for 5 min. and then at r.t. for 1.5 h, it was quenched with 2N HCl (1 mL). The solvent was removed and the residue was dissolved in acetone (15 mL). After adding Dowex-40 resin (2 g) the solution was stirred at r.t. for 20 min. The resin was filtered off and the solvent was removed. The residue was extracted with CH_2Cl_2. The organic layer was washed with water, dil. $NaHCO_3$ soln., water, and brine. After drying over anhyd. $MgSO_4$, the solution was filtered and evaporated to yield the crude product (250 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (95:5) gave 3α-(2'-propenyl)-3β-hydroxy-5β-pregnan-20-one (45 mg) as a first fraction. Further elution with the same solvent mixture yielded 3β-(2'-propenyl)-3α-hydroxy-5β-pregnan-20-one (85 mg), m.p. 119-120° C.

EXAMPLE 57

a. 3α-Hydroxy-17b-ethynyl-5α-androstane

3α-*tert*-butyldimethylsilyloxy-17b-ethynyl-5α-androstane (100 mg, 0.24 mmol) was dissolved in acetonitrile/THF (30 mL, 2:1 v/v) and cooled to 0° C. Aqueous hydrofluoric acid (48%, 2 mL, 60 mmol) was added

dropwise and the reaction solution was allowed to warm to room temperature. After 2 hours, the reaction solution was poured into water and extracted with CH_2Cl_2. The organic phase was washed with aqueous sodium bicarbonate, dried over $MgSO_4$ and evaporated *in vacuo* to give a white solid (69 mg). This solid was purified by flash column chromatography (hexane/acetone 12:1) to give a white solid (59 mg, 82%); mp: 158 - 160° C.

b. <u>3α-*tert*-butyldimethylsilyloxy-17b-ethynyl-5α-androstane</u>

Under a dry argon atmosphere, diisopropylamine (125 mL, 0.95 mmol, distilled from CaH_2) was dissolved in THF (2 mL) and the solution was cooled to 0° C. *n*-Butyllithium (2.5 M in hexane, 380 mL, 0.95 mmol) was added dropwise. After stirring at this temperature for 5 min., the solution was cooled to -78° C. 3α-*tert*-butyldimethylsilyloxy-5α-pregnan- 20-one 20-enol diethylphosphate (164 mg, 0.288 mmol) was dissolved in THF (1mL) and this solution was added dropwise to the previously prepared solution of lithium diisopropylamide (still at -78° C). The reaction mixture was allowed to warm to room temperature over 2.5 hours. Water (1 mL) was added, then the mixture was diluted with 75 mL ether. The organic phase was washed with 1 M HCl, water and aqueous sodium bicarbonate, dried over $MgSO_4$ and evaporated *in vacuo* to give a semi-solid residue (100 mg, 83%) which was used without further purification.

c. <u>3α-*tert*-butyldimethylsilyloxy-5α-pregnan-20-one 20-enoldiethylphosphate</u>

Under a dry argon atmosphere, lithium bis(trimethyl-silyl) amide (1.0 M solution in THF, 1.02 mL, 1.02 mmol) was diluted with THF (1 mL). The orange solution was cooled to -78° C. 3α-*tert*-butyldimethylsilyloxy-5α-pregnan-20-one (400 mg, 0.92 mmol) was dissolved in THF (1

mL) and added dropwise to the previously prepared solution of lithium bis(trimethylsilyl) amide (still at -78° C). After stirring at this temperature for 45 min., diethylchlorophosphate (140 mL, 0.97 mmol) was added dropwise and the resulting solution was allowed to warm to room temperature. Water (1 mL) was added and then ether. The organic phase was washed with 1N HCl, water and aqueous sodium bicarbonate, dried over $MgSO_4$ and evaporated *in vacuo* to give a yellow oil (394 mg) which was purified by flash column chromatography (hexane/acetone 10:1) to give a clear oil (164 mg, 31%).

d. <u>3α-*tert*-butyldimethylsilyloxy-5α-pregnan-20-one</u>

Under a dry argon atmosphere, 3α-hydroxy-5α-pregnan-20-one (1.1 g, 3.5 mmol) was dissolved in anhydrous DMF (17 mL). Imidazole (0.94 g, 13.8 mmol) was added and the mixture was stirred until all dissolved. *tert*-Butyldimethylsilylchloride (1.04 g, 6.91 mmol) was then added and the reaction mixture was stirred overnight. Ether (approx. 50 mL) was added and the organic phase was washed with 1N HCl, water and aqueous sodium bicarbonate, dried over $MgSO_4$ and evaporated *in vacuo* to give a white solid (1.93 g). This solid was purified by flash column chromatography (100% CH_2Cl_2) to give a white solid (1.16 g, 78%).

EXAMPLE 58

<u>3α,21-hydroxy-3β-methyl-5α-pregnan-20-one, 21-mesylate</u>

To a solution of 3α,21-hydroxy-3β-methyl-5α-pregnan-20-one (77 mg, 0.22 mmol) in pyridine (1 mL) at 0° C was added 29 mL (42 mg, 0.37 mmol) of methanesulfonyl chloride. After stirring cold for 2h. and 40 min., the reaction was poured into water and the resulting precipitate was collected. Flash chromatography (6" of silica in a 20 mm dia. column, eluted with a gradient from 0 to 5% EtOAc/hexane) gave 54 mg (57%) of the mesylate as a white solid.

EXAMPLE 59

21-Bromo-3β-ethynyl-3α-hydroxy-5β-pregnan-20-one

To a stirred solution of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one(2.5 g, 7.3 mmol) in 125 mL methanol at 0° C was added a few drops of HBr followed by slow dropwise addition of a solution of bromine (1.28g, 8.0 mmol) in 25 mL of methanol. The reaction was slowly warmed to 25° C, and upon complete decolorization the solution was added to ice water containing a small amount of $NaHCO_3$. The precipitate was filtered, dissolved in dichloromethane, dried with $MgSO_4$ and concentrated *in vacuo* to yield a crude foam. (2.837g) Flash chromatography on 6 in. of silica gel in a 5.5 cm column collecting 50 mL fractions and eluting with 1% acetone:CH_2Cl_2 resulted in 1.96g(64%) of the title compound as a foam.

EXAMPLE 60

3β-Chloroethynyl-3α-hydroxy-5β-pregnan-20-one

A solution of cis-1,2-dichloroethylene (194 mg, 0.16 mL, 2 mmol) in dry THF (7 mL) was treated under N_2 with n-BuLi (2.5M in THF, 4 mmol, 1.4 mL) at -10° C. The mixture was stirred at -30° C for 20 min and then at -5° C for 10 min. It was recooled to -30° C and a solution of 5β-pregnan-3,20-dione, 20-ketal (360 mg, 1 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min. The cooling bath was removed and the mixture was stirred at room temperature for 0.5 hr. NH_4Cl solution (3 mL) was added to quench the reaction. The solvent was removed and the residue was then dissolved in acetone (25 mL). After adding 2N HCl (10 mL) the solution was stirred at r.t. for 1 h. Sat. $NaHCO_3$ soln. was added to neutralize the acid. The solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water and then brine. After drying over anhyd. $MgSO_4$, the solution was filtered and evaporated to yield the crude product (427 mg). This crude product was then dissolved in a small

amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (95:5) gave the title compound as a colorless solid (130 mg).

EXAMPLE 61

3α-hydroxy-3β-methyl-5α-pregnan-20-one

To a solution (light yellow) of the (3R)-5α-pregan-3-spiro-2'-oxirane-20-one (101 mg, 0.305 mmol) and NaI (115 mg, 0.767 mmol) in anhydrous 1,2-dimethoxyethane (DME) (5mL) at room temperature was added η-Bu_3SnH (0.22 mL, 0.238 g, 0.818 mmol). The reaction solution became colorless. Azobisisobutylnitrile (AIBN) (10 mg, 0.061 mmol) was then added. The resulting solution was refluxed under a nitrogen atmosphere for 21 h. at which point TLC (3:1 hexane/acetone) indicated completion of the reaction. The reaction was quenched with methanol; the mixture was stirred at room temperature for a while. The solvent was removed in vacuo to give an oil which did not dissolve in either. Addition of CH_2Cl_2 gave a solution which was washed with water, 1N HCl, and saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to a white solid. Purification by gradient flash chromatography (hexane, 7:1 hexane/acetone, 5:1 hexane/acetone) furnished the titled compound (93mg, 92%).

It will be obvious to one skilled in the art that the above described compounds may be present as mixtures of diastereomers which may be separated into individual diastereomers. Resolution of the diastereomers may be conveniently accomplished by gas or liquid chromatography or isolation from natural sources. Unless otherwise specified herein, reference in the specification and claims to the compounds of the invention, as discussed above, is intended to include all isomers, whether separated or mixtures thereof.

Where isomers are separated, the desired pharmacological activity will often predominate in one of the

diastereomers. As disclosed herein, the biological activity of these compounds display a high degree of stereospecificity.

TBPS binding assays were conducted according to the following procedures, which are described in Gee, K.W.; Lawrence, L.J.; and Yamamura, H. I., "Modulation of the Chloride Ionophore by Benzodiazepine Receptor Ligands Influence of Gamma-Aminobutyric Acid and Ligand Efficacy," Molecular Pharmacology, 30:218, 1986. Male Sprague-Dawley rats (200-250g) were anesthetized with carbon dioxide and decapitated. The rats' brains were removed and the cerebral cortices were dissected and homogenized in 10 volumes of 0.32 M sucrose. Tissue was centrifuged at 1000 x g for 10 minutes and the resultant supernatant was centrifuged at 9,000 g for 20 minutes. The resultant pellet was resuspended in 10 volumes of 200 mM NaCl/50 mM Na-K phosphate pH 7.4 buffer (binding buffer) and then centrifuged at 9,000 x g for 10 minutes. This washing procedure was repeated twice and the final P2 pellet was resuspended in the binding buffer. For the binding assay, [^{35}S]TBPS (2 nM) was co-incubated in binding buffer with P2 membranes and 5 μM GABA and either 1) no additions to measure total binding; 2) 2 μM unlabeled TPBS to measure nonspecific binding; 3) the neurosteroid ranging in concentration from 1 nM to 10μM. Following a 90 minute incubation at 22°C, the suspension was filtered and the bound radioactivity was determined by liquid scintillation counting. The IC_{50}, which is the concentration of the neurosteroid producing a 50% inhibition of TBPS binding, was calculated using the non-linear curve fitting routine on Excel Solver by Microsoft. A lower IC_{50} indicates higher affinity for the steroid site on the $GABA_A$ receptor-chloride channel complex. Table 1 gives the IC_{50} values of representative steroids used in the present invention.

TABLE 1

Neuroactive Steroid	IC_{50} (nM)
3β-(4'-Acetylphenyl)ethynyl-3α-hydroxy-5α-pregnan-20-one	5
3β-(4'-Carboxyphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one ethyl ester	8
3β-(4'-Acetylphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	14
3α-hydroxy-21-(pyrid-4-ylthio)-5α-pregnan-20-one	23
3α-hydroxy-5β-pregnan-20-one	25
3β-(chloroethynyl)-3α-hydroxy-5β-pregnan-20-one	26
3α-Hydroxy-3β-methyl-5β-19-nor-pregnan-20-one	26
3α,20a-Dihydroxy-21-ethyl-5α-pregnane	27
3β-(4'-Dimethylaminophenyl)ethynyl-5β-pregnan-20-one	28
3β-azidomethyl-3α-hydroxy-5α-pregnan-20-one	34
3α,20a-Dihydroxy-21-methyl-5α-pregnane	35
3α-hydroxy-5α-pregnan-20-one	37
3β-ethynyl-3α-hydroxy-5β-pregnan-20-one	39
3α-hydroxy-3β-methoxymethyl-5β-pregnan-20-one	40
3β-(4'-biphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	43

Neuroactive Steroid	IC_{50} (nM)
3β-Ethynyl-3α-hydroxy-19-nor-5β-pregnan-20-one	44
3β-(1-Hexynyl)-3α-hydroxy-5β-pregnan-20-one	44
3β-ethenyl-3α-hydroxy-5β-pregnan-20-one	46
3α-Hydroxy-3β-(4'-nitrophenyl)ethynyl-5β-pregnan-20-one	46
3α-Hydroxy-3β-(4'-methyoxyphenyl) ethynyl-5β-pregnan-20-one	47
3β-(4'-Trifluoromethylphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	52
3α-hydroxy-3β-ethynyl-5α-pregnan-20-one	53
3α-Hydroxy-3β(E)-(2-phenylethenyl)-5α-pregnan-20-one	53
3α-Hydroxy-3β-(2'-propynyl)-5α-pregnan-20-one	56
3β-(4'-Chlorophenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	58
3α-Hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one	59
3α-hydroxy-3β-methyl-5α-pregnan-20-one	62
3β-(1-Octynyl)-3α-hydroxy-5β-pregnan-20-one	63
3β-(1-Heptynyl)-3α-hydroxy-5β-pregnan-20-one	65
3α-hydroxy-3β-methoxymethyl-5α-pregnan-20-one	67

Neuroactive Steroid	IC_{50} (nM)
3α-hydroxy-17b-cyano-5α-androstane	67
3β-(4'-cyanophenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	73
3α,21-dihydroxy-5α-pregnan-20-one 21-acetate	75
3α-Hydroxy-3β-(2'-propenyl)-5β-pregnan-20-one	75
3α-hydroxy-21-methyl-5β-pregnan-20-one	78
3α-Hydroxy-3β-(pentafluorophenyl) ethynyl-5β-pregnan-20-one	79
3α-Hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one	80
3α-Hydroxy-3β-(4'-methylphenyl) ethynyl-5β-pregnan-20-one	88
3α-hydroxy-3β,21-dimethyl-5α-pregnan-20-one	89
3α-Hydroxy-3β-(6-oxo-1-heptynyl)-5β-pregnan-20-one	90
3α-hydroxy-3β-methyl-5α-19-nor-pregnan-20-one	91
3α-hydroxy-3β-chloromethyl-5α-pregnan-20-one	93
3β-(3'-Bromo-1-propynyl)-3α-hydroxy-5β-pregnan-20-one	94
3α-hydroxy-21-methoxymethyl-5β-pregnan-20-one	96
3α,20a(S)-dihydroxy-5α-pregnane	97

Neuroactive Steroid	IC_{50} (nM)
3α-Hydroxy-3β-(phenylethynyl)-5β-pregnan-20-one	98
3β-Benzyl-3α-hydroxy-5β-pregnan-20-one	109
3β-(2,4-Difluorophenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	109
3α,21-dihydroxy-5α-pregnan-20-one 21-hemisuccinate	117
3α,21-dihydroxy-5β-pregnan-20-one 21-acetate	119
3α-Hydroxy-3β-(2'-phenylethyl)-5β-pregnan-20-one	135
3β-ethyl-3α-hydroxy-5β-pregnan-20-one	136
3α-Hydroxy-3β-(3'-hydroxypropynyl)-5β-pregnan-20-one	137
3α-Hydroxy-3β-[3'(RS)-hydroxybutynyl]-5α-pregnan-20-one	140
3α,20-dihydroxy-20-methyl-5α-pregnane	151
3α-Hydroxy-3β-[4'(R/S)-hydroxypentynyl]-5β-pregnan-20-one 4'(R/S)-hemisuccinate sodium salt	153
3β-(Ethoxymethyl)-3α-hydroxy-5α-pregnan-20-one	154
3α-Hydroxy-3β-methyl-5β-19-nor-pregn-17(Z)-ene	154
3β-(5'-Cyanopentynyl)-3α-hydroxy-5β-pregnan-20-one	158
3α,20a-dihydroxy-3β-methyl-5α-pregnane	166

Neuroactive Steroid	IC_{50} (nM)
3β-(4'-Acetoxyacetylphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	171
20,20-ethylenedioxy-3α-hydroxy-5α-pregnan-20-one	176
3α-Hydroxy-3β-[4'(R/S)-hydroxypentynyl]-5α-pregnan-20-one	178
3α,21-dihydroxy-5β-pregnan-20-one (5β-THDOC)	180
3α-hydroxy-5β-pregnan-cis(Z)-17(20)-ene	180
3β-(Bromomethyl)-3α-hydroxy-5α-pregnan-20-one	190
3β-(2'-Hydroxyphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	192
3α-hydroxy-3β-fluoromethyl-5α-pregnan-20-one	200
3α,20b-dihydroxy-5β-pregnane	206
3α-Hydroxy-5β-19-nor-pregn-17(z)ene	209
3α,21-dihydroxy-3β-methyl-5α-pregnan-20-one 21-Hemisuccinate, sodium salt	211
3α,21-Dihydroxy-3β-ethynyl-5β-pregnan-20-one	216
3α-hydroxy-21-methyl-5α-pregnan-17(20)(Z)-ene	217
3α,21-dihydroxy-5β-pregnan-20-one 21-hemisuccinate	224
3α-hydroxy-3β-(2'-methoxyphenyl) ethynyl-5β-pregnan-20-one	238

Neuroactive Steroid	IC_{50} (nM)
3α,21-dihydroxy-3β-methyl-5α-pregnan-20-one 21-Hemisuccinate	241
3β-(But-3-enyl)-3α-hydroxy-5α-pregnan-20-one	242
3α,21-dihydroxy-5α-pregnan-20-one 21-hemisuccinate, sodium salt	251
3α-Hydroxy-3β-trifluoromethyl-19-nor-5α-pregnan-20-one	251
3α,21-Dihydroxy-3β-ethynyl-5β-pregnan-20-one, 21-acetate	251
3α-hydroxy-21-methoxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one	252
3α-Hydroxy-5α-androstane	252
3α-Hydroxy-17b-ethynyl-5α-androstane	254
3α,21-dihydroxy-3β-methyl-5α-pregnan-20-one 21-mesylate	255
3α,20-dihydroxy-3β,20-dimethyl-5α-pregnane	264
3α-hydroxy-3β-ethyl-5α-pregnan-20-one	265
3α,21-Dihydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one	265
3β-[(3',4'-Dimethoxyphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one	283
3α,20a-dihydroxy-5β-pregnane	292
3β-(Buta-2,3-dienyl)-3α-hydroxy-5α-pregnan-20-one	347

Neuroactive Steroid	IC_{50} (nM)
3α-hydroxy-17(Z)-methoxymethylene-19-nor-5α-androstane	350
3α-hydroxy-3β-methyl-5α-19-nor-pregn-(Z)-17(20)-ene	364
3α,21-Dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one, 21-acetate	370
3α-hydroxy-19-nor-5α-pregnan-cis-17(20)-ene	373
3α-hydroxy-3β-phenyl-5β-pregnan-20-one	382
3β-(3'-Hydroxyphenyl)ethynyl-3α-hydroxy-5β-pregnan-20-one	407
3β-cyanomethyl-3α-hydroxy-5α-pregnan-20-one	439
3α-hydroxy-17b-hydroxymethyl-5α-androstane	455
3β-Ethynyl-3α-hydroxy-5β-19-nor-pregn-17(z)-ene	460
3α-Hydroxy-3β-trifluoromethyl-5β-19-nor-pregn-17(20)-ene	482

The compounds of the invention may be prepared by known techniques. For example, the naturally occurring metabolites of progesterone may be extracted from various animal excretion sources, e.g., urine, or extracted from vegetable products like soy or yams. Such extractions are conducted using the following steps: (i) hydrolysis with HCl; (ii) extraction with toluene; (iii) removal of acidic material from the toluene extract; (iv) elimination of substances other than pregnanes from the neutral toluene-soluble fraction by precipitations from ethanolic solution with dilute NaOH and with water; and (v) weighing of the purified pregnanes obtained. See Marrian et al., "The Isolation of Pregnane-3α-ol-20-one," Biochem., 40:376-380 (1947). These extracted compounds may then be chemically altered to form the desired derivatives, or used directly.

The pharmaceutical compositions of this invention are prepared in conventional dosage unit forms by incorporating an active compound of the invention, or a mixture of such compounds, with a nontoxic pharmaceutical carrier in nontoxic amounts sufficient to produce the desired pharmacodynamic activity in a subject, animal or human. Preferably, the composition contains the active ingredient in an active, but nontoxic amount, selected from about 5 mg to about 250 mg of active ingredient per dosage unit. The compositions and methods of this invention prevent insomnia and produce sleep.

The pharmaceutical carrier employed may be, for example, solids, liquids, or time release substances (see, e.g., Remington's Pharmaceutical Sciences, 14th Edition, 1970). Representative solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid, microcrystalline cellulose, polymer hydrogels, and the like. Typical liquid carriers are propylene glycol, aqueous solutions of β-cyclodextrins, syrup, peanut oil, olive oil and like emulsions. Similarly, the carrier or diluent may include

any time-delay material well known to the art, such as glycerol monostearate or glycerol distearate alone or with wax, microcapsules, microspheres, liposomes, or hydrogels.

A wide variety of pharmaceutical forms can be employed. Thus, when using a solid carrier, the preparation can be plain milled, micronized, in oil, tableted, placed in a hard gelatin or enteric-coated capsule in micronized powder or pellet form, or in the form of a troche, lozenge, or suppository. When the compounds of the invention are administered in the form of suppositories for rectal administration, the compounds may be mixed in material such as cocoa butter and polyethylene glycols or other suitable non-irritating materials solid at room temperature, but liquid at rectal temperature. When using a liquid carrier, the preparation can be in the form of a liquid, such as an ampule, or an aqueous or nonaqueous liquid suspension. Liquid dosage forms will generally also contain pharmaceutically acceptable preservatives and the like. In addition, parental administration, nasal spray, sublingual and buccal administration, and timed release skin patches are also suitable pharmaceutical forms for topical administration of the compounds of the invention.

The preferred formulations are for oral administration. The inventors have discovered that specific formulations can significantly alter the activity of the neuroactive steroid, which tends to be insoluble in water and therefore poorly absorbed after oral administration. For example, it was thought that pregnanolone was orally inactive. L. Gyermek, "Pregnanolone: a Highly Potent, Naturally Occurring Hypnotic-Anesthetic Agent" Proc. Soc. Exp. Biol. Med. 125:1058-1062, 1967.

The inventors have discovered various formulations of pregnanolone which the body can absorb through the gastrointestinal tract in sufficient quantities to induce sleep after oral administration. The bioavailability of a drug is quantified by measuring serum blood levels before and

after administration. This method of quantification is described by Robert H. Purdy et al. in "Radioimmunoassay of 3α-hydroxy-5α-pregnan-20-one in rat and human plasma" Steroids 55:290-296 (1990), which is incorporated by reference in its entirety. Generally, after a blood sample is drawn, the serum is separated and then frozen. The serum is chromatographed to remove competing materials including endogenous steroids. Pregnanolone has a retention time of 19.0 minutes when it is chromatographed using HPLC techniques on an 8 cm Radial-Pak-5-μm silica column (Waters Associates) using the solvent system 0.2% ethanol (95%) in dichloromethane at a flow rate of 3 ml/min. Then, a radioimmunoassay is run to quantify the serum blood levels of the chromatographed material.

To demonstrate that pregnanolone, when administered orally in certain formulations, is orally active to induce sleep in humans, the inventors conducted a trial using 8 healthy male volunteers between the ages of 18 and 35. The study was an unblinded, single dose crossover study in which subjects were sequestered prior to and immediately following drug administration.

Baseline medical history, laboratory values, drug and alcohol screens were done for each subject. Throughout the study the subjects remained in the clinical facility from Day-1 until 48 hours post drug administration. While in the clinic, the ingestion of caffeine, alcohol, or other drugs was prohibited to the subjects.

Following an overnight fast, a defined high fat breakfast was served to each subject and consumed within 15 minutes. Starting at 0900 GMT the subjects were administered pregnanolone according to the randomization defined in the protocol. Pregnanolone (500 mg; granulated powder) was administered orally in one of four formulations. Formulation 1 had the following ingredients: pregnanolone (micronized, obtained from AKZO, Arnheim, The Netherlands) (5%), polyvinylpovidone (polyvinylpyrrolidone

or PDP) (Luviskol K-30, from BASF, Germany) (94%), and sodium lauryl sulfate (SLS) (from Nomeco, Copenhagen, Denmark) (1%). Formulation 2 contained preganolone (12.3%), and β-cyclodextrin (7 moles of H_2O per 1 mole of cyclodextrin; from Sigma, St. Louis) (87.7%). Formulation 3 contained pregnanolone (99%; nanosized), and sodium lauryl sulfate (1%). Formulation 4 contained pregnanolone (99%), and sodium lauryl sulfate (1%). The pregnanolone used in the study was uniformly micronized (available from AKZO) in all cases with the exception of Formulation 3 which was nanosized (submicron particle size). Access to water ad libitum was allowed except during the hour immediately following drug administration. Meals were standardized in time throughout the study.

Formulation 1 was prepared by dissolving all constituents in ethanol. The mixture was stirred and heated until most of the ethanol had evaporated. The product was dried to completion in vacuum for 24 hours and ground in a mortar.

Formulation 2 was prepared by shaking 1.23 g. of pregnanolone, 6.77 g. cyclodextrin in 50 ml. of water for hours at 37°C. The complex was filtered, dried under vacuum for 24 hours, and ground in a mortar.

Formulation 3 was prepared by dissolving the constituents in carbon dioxide at an elevated temperature and high pressure (up to 700 bar). The pressure is released and the particles precipitate, which produced some submicron particles.

Formulation 4 was prepared by dry blending the micronized pregnanolone with sodium lauryl sulphate, followed by sieving.

Serum samples for analysis of pregnanolone level were obtained prior to and immediately following drug administration and continued for 24 hours. Serum was separated, frozen, and stored until analyzed by radioimmunoassay.

Samples obtained during the study were analyzed for pregnanolone using radioimmunoassays and the results analyzed using appropriate non-compartmental pharmacokinetic parameters. Relative bioavailability assessments were made between formulations based on observed serum concentration of pregnanolone.

Evaluation of clinical response to drug administration was obtained through observations carried out by the clinical facility's staff and trained observers. Observations regarding onset of and apparent depth of sleep were recorded over the course of the four hour treatment period and later correlated with serum pregnanolone concentrations. Duration of sleep was not evaluated as subjects were frequently disturbed in the process of obtaining vital signs and blood samples.

Each individual received the four formulations separated by drug-free wash-out days during the three week trial. No serious or unexpected adverse effects were noted for any of the treatment groups. Body temperature was closely monitored and recorded prior to drug administration at 0.5, 1.0, 1.5, 2.5, 3, 4, 8, and 12 hours post drug administration. No decrement in body temperature was observed in any of the subjects during the course of the study with any of the formulations.

After approximately one hour signs of drowsiness and/or sleep was observed. Among the volunteers receiving pregnanolone in β-cyclodextrin (Formulation 2), seven of eight subjects were observed to sleep with time of onset ranging from <1 hour to 3 hours post drug administration. Duration of sleep was impossible to evaluate as subjects were awakened every 30 minutes for blood draws and vital signs. Subjective evaluations indicated depth of sleep ranging from light to difficult to arouse. Results are summarized in Figure XVI where arrows mark points at which observations of sleep were recorded. Only one subject was observed to remain awake throughout the study period after

receiving pregnanolone formulated in β-cyclodextrin. Figure XVI also shows the plasma blood levels for this formulation. Peak plasma levels for the 8 volunteers ranged from about 40 to 120 nanograms per ml. Table 2 shows that the mean peak concentration for this formulation was 61.7 nanograms per ml.

Table 2
Relative Absorption of Four CCD 3045 Formulations

CCD 3045 Formulation	AUC (ng.h/mL)	Peak Concentration (ng/mL)
PVP Formulation	136.2 (61.1)*	60.9 (28.7)*
Beta CD Formulation	138.4 (53.8)	61.7 (28.5)
Nanosized Powder + 1% SLS	59.8 (23.8)	22.0 (9.9)
Micronized Powder + 1% SLS	117.1 (36.6)	59.4 (21.0)

* Mean (SD)

Similar findings were observed in subjects receiving pregnanolone formulated with polyvinylpovidone and sodium lauryl sulfate. All eight subjects were observed to exhibit signs of hypnosis at time points ranging from <1 hour to three hours. Fig. XV shows that the peak plasma levels for this formulation ranged from about 20 to 110 nanograms per ml. Table 2 shows that the mean peak concentration for the PVP formulation was 60.9 nanograms per ml.

Formulation 4 consisting of micronized pregnanolone with 1% sodium lauryl sulfate produced peak plasma levels ranging from about 40 to 110 nanograms per ml. Table 2 shows that the mean peak concentration for the micronized formulation was 59.4 nanograms per ml.

Formulation 3 produced peak plasma levels ranging from 10 to 35 nanograms per ml. Table 2 shows that the mean

peak concentration for the nanosized formulation was 22.0 nanograms per ml.

The assessments of relative absorption or bioavailability demonstrate that all but the nanosized formulation have comparable absorption both in terms of the rate and extent of absorption. The serum samples were analyzed for each subject over a 48 hour interval. Figure XIX illustrates the mean serum levels observed for the four different formulations. Three of the formulations, PVP, Beta CD, and the Micronized Powder + SLS all had comparable mean serum concentration-time profiles, while the nanosized pregnanolone formulation had much reduced levels, its peak approximately 35% of the others. In addition to examining the mean serum levels, relative assessment of the peak and extent of absorption can be determined by looking at the peak concentration and area under the serum concentration-time (AUC) profile. Table 1 illustrates those findings.

Figures XV-XVIII are plots of the serum concentration versus time for individual subjects. At each point where sedative effects were observed, an arrow is placed at the point in time. As can be observed, higher serum concentrations, generally above 30 ng/ml, are related to observe sedative effects.

In another human study, plasma concentrations and sleep propensity were measured in 18 young, healthy, male volunteers for 2 hours after oral administration of single doses of pregnanolane. Two different formulations were tested. Formulation I used β-cyclodextrin as the excipient. Formulation II used sodium lauryl sulfate as the excipient. Both β-cyclodextrin and sodium lauryl sulfate are known pharmaceutical excipients and were chosen to enhance the absorption of pregnanolone without affecting the toxicological profile of the compound.

Formulation I was a β-cyclodextrin inclusion complex prepared by suspending 15.0g of pregnanolone and 82.57 g β-cyclodextrin in 610 mL distilled water in a 1000 mL

Erlenmeyer flask. The suspension was stirred by magnetic bar stirrer for 36 hours at 37°C. The suspension was then filtered through a 0.22 μm filter (Millipore, type GV) on which the complex was collected. The resultant complex was dried in a drying cupboard at 50°C for 12 hours after which the mass was comminuted in a porcelain mortar, and the granulate was passed through a 300 μm sieve. The granulate was dried in vacuo over P_2O_5 at 50°C for 24 hours. This formulation was packaged in sachets containing 150 mg of pregnanolone.

Formulation II was a 99:1 physical mixture of pregnanolone and sodium lauryl sulphate prepared by mixing 49.50 g pregnanolone, which had been passed through a 125 μm sieve, and 0.50 g sodium lauryl sulfate in a mortar and pestle for 30 minutes. The mixture is then passed through a 300 μm sieve. This formulation was packaged as 250 mg pregnanolone capsules and 500 mg pregnanolone sachets.

Prospective subjects were examined 21 days prior to their first dose to determine medical histories and baseline clinical laboratory values. The subjects were told not to take any prescription or non-prescription drugs before or during the study. Each subject participated twice within a two-week period. Successive single dose treatments were separated by a one-week interval.

Test subjects fasted overnight and reported to the study center 3 hours prior to dose administration. A light snack was given 2 hours prior to the dose administration. A standard FDA-approved high-fat breakfast was given to the subjects 20 minutes prior to dosing and was finished 5 minutes prior to dosing. After breakfast, the subjects were given one of the two formulations in liquid yogurt. The contents of a sachet was added to 100 mL of liquid yogurt and stirred for 20-30 seconds. The subjects then drank the yogurt. An additional 30 mL of yogurt was mixed with the remnants in the cup and consumed assure full administration. After drinking this, the sides and bottom

of the cup were scraped and fed to the subject. No liquids were allowed for the first hour after drug administration. Subjects had to remain sitting or standing for the first hour after ingestion.

Mean plasma concentrations for the β-cyclodextrin formulation reached a peak value of 82.08 $\pm$ 57.18 ng/mL at 1 hour post dosing. The mean plasma concentration was 79.33 $\pm$ 49.71 ng/mL at 1.5 hours and was 74.21 $\pm$ 37.21 ng/mL at 2 hours.

The sodium lauryl sulfate formulation reached its peak mean plasma concentration later. The mean value was 64.06 $\pm$ 54.91 ng/mL of 1.5 hours and was 66.63 $\pm$ 49.60 ng/mL at 2 hours post dosing. These statistics are shown in Figure XX.

Subjects were monitored by EEG, EOG, and EMG which were recorded for 10 minutes in a darkened room with closed eyes. The recordings were made for 5 minutes under reaction time conditions. Subjects were supposed to respond as quickly as possible to one of two different tones presented in random sequence and intervals by pressing a button with their left or right thumb. The subjects then were recorded for 5 minutes without interruption.

As shown in Figure XXI the time awake decreased post dosing. The number of sleep attempts was also determined. A sleep attempt was defined as a transition from wakefulness to any sleep stage within the 5 minute recording session. On each study day, a subject had five polygraphic recording sessions. Therefore, the maximum number of sleep attempts could vary from 0 to 5. A five indicates that sleep was observed even during the 5 minute pre-dose recording session. The number of sleep attempts was plotted against the maximum plasma concentration (C max) in Figure XXIIA. A low plasma concentration generally coincided with few sleep attempts while high plasma concentrations coincided with a high number of sleep attempts. Figure XXIIB

shows the trimean plasma concentration levels for different numbers of sleep attempts.

The data from these human studies show: (1) that pregnanolone can be formulated in such a way as to produce suitable blood levels from oral administration; and (2) that the blood levels of pregnanolone found in the volunteers correlated to the observation of drowsiness and/or sleepiness.

To demonstrate the pharmacological properties of the compounds used in the present invention, the inventors had in vivo experiments run on rats in which the effects of pregnanolone, 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, 3α-hydroxy-3β-methyl-5α-pregnan-20-one, 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate sodium salt, 3α-hydroxy-3β-ethynyl-5α-pregnan-20-one, 3α,21-dihyrdoxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate, 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one, 3α-hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one, 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one 21-phosphate disodium salt on brain EEG, locomotor activity, and body temperature were measured. Rats are a suitable model for human sleep because all compounds that are hypnotics in man have hypnotic effects in rats and vice versa. Edgar, D.M. "Drug Discovery and Evaluation Using the Advanced Technology Sleep-Wake Bioassay Laboratory at Stanford University" p. 4, December 1992. In addition, the rat model for human sleep has been accepted by peer-review committees at NIH Id. at 5. Furthermore, rat sleep and human sleep are fundamentally the same. Id. at 4. For example, the proportion of time spent in NREM versus REM sleep is about 4:1 in both rats and humans. Id. at 5.

Rat preparation, monitoring, and analysis of the data was conducted as described below. Adult, male Wistar rats (275-350 g at the time of surgery, Charles River

Laboratories) were anesthetized (Nembutal, 60mg/kg) and surgically prepared with a cranial implant that permitted intermittent EEG and EMG recording. Body temperature and locomotor activity were monitored via a miniature transmitter (Minimitter) surgically placed in the abdomen. The cranial implant consisted of stainless steel screws (2 frontal [+3.9 AP form bregma, ±2.0 ML] and two occipital [-6.4 AP, ±5.5 ML]) for EEG recording. Two Teflon-coated steel wires were positioned under the nuchal trapezoid muscles for EMG recording. All leads were soldered to a miniature connector prior to surgery, and chemically sterilized in Glutarex (3M Co.). The implant assembly was affixed to the skull with dental acrylic. A minimum of three weeks was allowed for recovery.

Each rat was permanently housed in its own individual recording cage located within separate, ventilated compartments of custom-designed stainless steel cabinets. Each Nalgene microisolator cage, which is an individual recording cage, was enhanced with a filter-top riser and swivel-commutator. Food and water were available <u>ad libitum</u>. A 24 hour light-dark cycle (12 hours on, 12 hours off) was maintained throughout the study using 4-watt fluorescent bulbs 5cm from the cage. Animals were undisturbed for three days both before and after treatments. In practice, animals were undisturbed during the weekend. Treatments were administered on Monday evening or Tuesday morning, and the animals were again left undisturbed until Friday, when cages were cleaned and the data copied off the computers.

The computers monitored the EEG, EMG, body temperature and non-specific locomotor activity (LMA) via telemetry, and drinking activity from 48 rats simultaneously. The computer classified the arousal state every 10 seconds using pattern-matching algorithms which included behavior-dependent contextual rules. Drinking and LMA were recorded every 10 seconds, while body temperature was recorded every minute.

As nocturnal creatures, rats are most active when the lights are off, and like humans, are most sensitive to hypnotic effects toward the end of their activity dominated period. Therefore, time of treatment is important. By administering the drug at CT-18, which is the peak of the rat's activity dominated period, the natural tendency for the rats to sleep at lights on (3:00 clock time or Circadian Time, CT-0) cannot mask the hypnotic activity of the drug. By administering the drug at CT-5 when the propensity for REM sleep is greatest, the drug's effect on REM sleep can be measured more accurately. The activity dominated period begins when the lights are turned off at CT-12 or 15:00 clock time on the Figures.

Compounds were suspended in sterile 0.25% methylcellulose, 20-50% hydroxy propyl cyclodextrin, or water depending on the physical properties of the steroid. These vehicles provide adequate bioavailability for all hypnotics tested to date. The drugs and controls were injected intraperitoneally or administered orally in a volume of 1 ml/kg or 10 mg/kg, respectively, under dim red illumination for the CT-18 studies or under regular light for the CT-5 studies.

A matched control group, N=20, was selected from a pool of 60 available control candidates. These matched control groups were constructed as follows. For each of the variables (NREM, REM, LMA, body temperature, and sleep bouts), a curve of hourly-averaged values was computed for each candidate for the 24 hours preceding injection. The 20 candidates whose curve best fit (least squares) the mean curve for the treatment group were selected. Therefore, a matched-pairs procedure was not used, but parallel treatment groups with closely comparable pre-treatment baseline data were used.

Figures IA, IB, and IC demonstrate that pregnanolone administered at either CT-5 or CT-18, is effective in promoting NREM sleep independent of the time at which it

was administered. At both times, there is rapid onset and high potency. At CT-5, the rat is more rested and it is more difficult to induce sleep. These results indicate that the neuroactive steroids of the invention are desired hypnotic agents when sleep must be induced earlier than usual, as may occur in shift work and jet lag. Furthermore, since a physician prescribing a hypnotic can never be certain of the patient's sleep debt, it is advantageous to have a hypnotic drug such as the present steroids with a predictable hypnotic action independent of the patient's sleep debt.

Figures IA-C also indicate that pregnanolone lacks a rebound effect. Rebound effect is defined as the reduction of NREM sleep after the hypnotic action of the treatment has returned to control levels. Pregnanolone produced no rebound reduction of NREM sleep at CT-5 or CT-18 for any dose. As shown by FIG. XXIVA, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one also produced no rebound reduction of NREM sleep at CT-18. Triazolam showed a rebound reduction of NREM sleep when administered at CT-18. Figure VIB shows a star ("*") at the right hand side, indicating a statistically significant reduction in NREM sleep.

Pregnanolone is unique among hypnotic agents in being both potent at both CT-5 and CT-18, and not producing rebound wakefulness. While our studies indicate that Zolpidem did not produce rebound insomnia, it was also unable to effectively induce NREM sleep at CT-5, as shown in Figure VIIIA. This feature could possibly imply that pregnanolone or other neuroactive steroids which do not produce rebound insomnia would produce less tolerance, withdrawal, and rebound insomnia clinically compared to current BZ-receptor ligands, which are their most important limitations.

Figures IIA, IIB, IIC, and XXVA demonstrates that administration of pregnanolone and the administration of 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one causes a strong

increase in the duration of uninterrupted NREM sleep or "sleep bout length". Sleep bout length is of interest because it may parallel the human tendency to awaken periodically during the night, which has been shown to be an important factor in determining the restorative value of sleep in humans. This strong consolidation of sleep has great potential in the treatment of age related insomnia.

Figures IIIA, IIIB, and IIIC indicate that pregnanolone tends not to affect body temperature, contrary to the teachings of Attallah Kappas et al. who found that intramuscular administration of pregnanolone caused a pyrogenic effect in man. "Fever-Producing Steroids of Endogenous Origin in Man" 105:68/701-68/708 <u>A.M.A. Archives of Internal Medicine</u> 1960. Note in the hours prior to injection, body temperature fluctuates in a near-sinusoidal rhythm. Handling and injection cause a brief increase in body temperature. Figure XXVIA also shows that 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one has a minimal effect on body temperature. Triazolam and Zolpidem affect body temperature as revealed in Figures VIE and VIIID, respectively. A lack of temperature effect suggests that the NREM inducing mechanism of pregnanolone and the neuroactive steroids of the invention does not produce undesirable side-effects on homeostatic control functions.

Pregnanolone produces a moderately smaller reduction in locomotor activity (LMA) over time at 30 mg/kg and no reduction at 10 mg/kg as compared to Triazolam and Zolpidem, as illustrated in Figures IVA-C, VIF and VIIIE. 3β-Ethynyl-3α-hydroxy-5β-pregnan-20-one also produced a moderately smaller reduction in LMA over time as illustrated in Figure XXVIB. Both Triazolam and Zolpidem reduced LMA below vehicle control levels when administered at CT-5 as indicated in the bar graph of Figure XII, even though Triazolam had no hypnotic effect at CT-5. Figure XIV demonstrates that during the effective period of pregnanolone and Zolpidem when the rats are asleep, they

are not moving. However, Zolpidem continues to depress LMA even after the hypnotic action has worn off. Motor incoordination appears to be comparatively reduced, which is a valuable trait for hypnotics used with the elderly.

Figures VA-VC and XXIVB indicate that pregnanolone and 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, respectively, lack an inhibitory effect on REM sleep compared to other hypnotics, such as the benzodiazepines, which reliably reduce REM sleep. Figures VIF and VIIIF show that Triazolam and Zolpidem, respectively, administered at CT-18 reduced REM sleep in the first few hours after administration. For example, the bar graph in Figure XIII demonstrates that Zolpidem interferes with REM sleep at a dose administered at CT-5 that induced significantly less NREM sleep than pregnanolone.

Pregnanolone, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, and the other neuroactive steroids of the invention are unique in that they combine a potent NREM-promoting effect with slight REM-interference. Strong inhibition of REM sleep in humans, such as alcohol and barbiturates produce, has been associated with sleep disruption as the drug effect diminishes and REM rebound sets in. Although the biological function of REM sleep is unknown, the finding that the neuroactive steroids of the invention only slightly interfere with REM, while potently inducing sleep, is a favorable attribute.

Pregnanolone produced a high-frequency, highamplitude EEG pattern during NREM sleep. This EEG pattern is reproduced in Figure VIIA. In contrast, deep normal NREM sleep generally has a low frequency, high amplitude EEG wave. Pregnanolone's effect on EEG spectral profiles was closely similar after both CT-5 and CT-18 treatments. This EEG pattern was distinct from the other hypnotics, as shown in Figure VIIB.

As illustrated in Figures XA pregnanolone had characteristic effects on NREM EEG delta activity (power).

The "depth" of NREM sleep may be quantified as the proportion of EEG activity that occurs at low (delta) frequencies of 0.1-4.0 Hz. For each 10 second period of EEG activity that was determined to be NREM sleep, the EEG delta power was measured and averaged across 20 minute intervals and then compared to vehicle controls. Figure XA indicates that pregnanolone reduced the percentage of EEG power in the low delta range for at least 5 hours after administration. In contrast, Figures XC and XB show that Triazolam and Zolpidem, respectively, increased the percentage of EEG power in the delta range for at least 1 hour after treatment.

It was also found that pregnanolone produced a characteristic distribution of frequencies in the EEG during the period of peak drug effect. The purpose of determining a drug's "EEG spectral profile" is to determine which frequencies have increased or decreased in abundance following treatment. Figure XIA shows that pregnanolone shifted the power from the lower frequencies (0.1-8 Hz) to the higher frequencies (≥10 Hz). In contrast, Zolpidem and Triazolam increased the percentage of EEG power in the lower delta frequency range. See Figures XIB and XIC, respectively.

Using the same experimental design as used for pregnanolone, nine other neuroactive steroids with various structure modifications of the invention were tested on rats. All nine synthetic neuroactive steroids produced profiles similar to pregnanolone. The effects on REM, NREM, rebound insomnia, locomotor activity, body temperature and power spectrum were similar to those described above for pregnanolone and 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one. The changes in REM, rebound insomnia, and NREM sleep in these experiments are summarized in Figures XXIIIA-C, respectively. Like pregnanolone, all nine steroids showed only slight REM interference, while producing a potent NREM promoting effect. (Figs. XXIIIA & C).

Note also that these steroids produced little or no rebound effects. See Fig. XXIIIB.

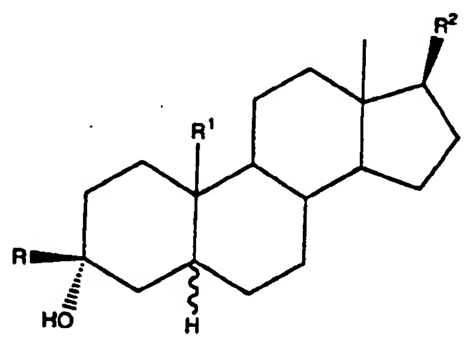
In summary, pregnanolone and the nine other synthetic neuroactive steroids strongly increased NREM sleep, but did not cause any temperature drop, only slightly interfered with REM sleep, did not significantly reduce locomotor activity after the hypnotic action had worn off, and was not followed by rebound wakefulness. By contrast, Zolpidem and Triazolam reduced body temperature and locomotor activity after the hypnotic effects had worn off. Zolpidem and Dexmedetomidine (an antihistamine which is a class of drugs known to cause drowsiness) strongly interfered with REM sleep. Dexmedetomidine produced strong rebound effects. Taken together, these results show that the neuroactivity steroids of this invention may have a more specific mode of hypnotic action than BZ-receptor ligands, thereby producing less impairment of other important functions (i.e., body temperature, and possibly motor coordination or motivation). Benzodiazepines, in contrast, impair LMA after the hypnotic effects wear off, a trait that is undesirable in a sleep inducing medication.

The method of producing sleep, in accordance with this invention, comprises administering to a subject in need of induced sleep, a compound of the invention, usually prepared in a composition as described above with a pharmaceutical carrier, in a nontoxic amount sufficient to produce sleep. The preferred dose is 5 mg to 250 mg of active compound.

While the preferred embodiments have been described and illustrated, various substitutions and modifications may be made thereto without departing from the scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustration and not limitation.

Claims

1. A method of inducing sleep comprising administering to an animal subject an amount of a 3α-hydroxy-3β-R-5α or 5β-19-R^1-17β-R^2-pregnane or androstane that will increase the duration of uninterrupted NREM sleep, and will maintain the level of locomotor activity found in normal sleep, said pregnane or androstane having the structural formula I:



I

wherein:

R is hydrogen, a lower alkyl group, a lower alkynyl group, a lower trihaloalkyl group, a lower monohaloalkyl group, a lower alkenyl group, and aryl group, or an aralkyl group;

R^1 is hydrogen or methyl;

R^2 is methylene (=CH_2), cyano, hydroxymethyl, methoxymethylene (=$CHOCH_3$), acetyl, 2'-hydroxyacetyl, 2'-hydroxyacetyl acetate, 1-hydroxyethyl, 2'-hydroxyacetyl hemisuccinate, 2'-methoxyacetyl, pyrid-4-ylthioacetyl, ethylene (=$CHCH_3$), propylene (=$CHCH_2CH_3$), 2'-hydroxyacetyl hemisuccinate sodium salt, 1-hydroxybutyl, and 1-hydroxy-1-methylethyl, 1-hydroxypropyl, 1-propionyl, 3-methoxypropionyl, ethynyl, 2'-mesyloxyacetyl, or 1'-(ethylenedioxy)ethyl;

and pharmaceutically acceptable 3-esters, 20-esters, 21-esters, 3,20-diesters, and 3,21-diesters, thereof;

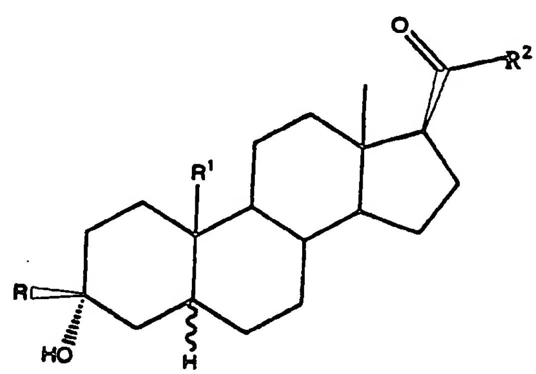
with the proviso that the steroid is not 3α,21-dihydroxy-5α-pregnan-20-one.

2. The method of claim 1 wherein the level of REM sleep is maintained compared to that found in normal sleep.

3. The method of claim 1 wherein substantial rebound insomnia is not induced.

4. The method of claim 1 wherein R is not hydrogen, the compound has an IC_{50} value of 5-300 nM in TBPS binding assays, and the compound is orally active.

5. The method of claim 1 wherein the compound has the structural formula II:



II

wherein:

R is a lower alkyl group, a lower alkynyl group, or a trihalo(lower) alkyl group;

R^1 is hydrogen or a lower alkyl group;

R^2 is hydrogen, hydroxy, a pyrid-4-ylthio group, a hemisuccinoyloxy group, or a phosphoryloxy group or the sodium salts thereof; with the provisos that:

(1) when R is hydrogen, R^2 is a pyrid-4-ylthio group;

(2) when R^1 is hydrogen, R is a trihalo(lower) alkyl group; and

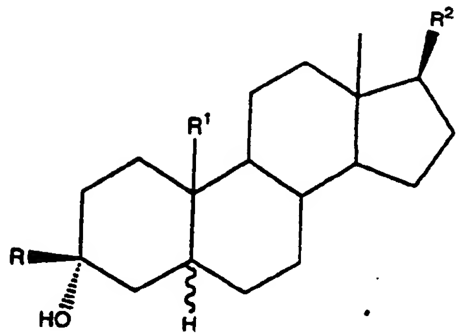
(3) when R^2 is other than hydrogen or a pyrid-4-ylthio group, R is a trihalo(lower) alkyl group.

6. The method of claim 5 wherein R is selected from the group consisting of lower alkynyl, and trihalo(lower) alkyl.

7. The method of claim 5 wherein R^2 selected from the group consisting of hemisuccinoyloxy, and phosphoryloxy and their sodium salts.

8. The method of claim 5 wherein the compound is selected from the group consisting of 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, 3α-hydroxy-3β-methyl-5α-pregnan-20-one, 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate sodium salt, 3α-hydroxy-3β-ethynyl-5α-pregnan-20-one, 3α,21-dihyrdoxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate, 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one, 3α-hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one, and 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one 21-phosphate disodium salt.

9. A method of modulating the $GABA_A$ receptor complex comprising administering to an animal subject an amount of a 3α-hydroxy-5-reduced steroid or derivative thereof that will increase the duration of uninterrupted NREM sleep, and will maintain the level of motor function and motivation found in normal sleep, said steroid having the structural formula I:



I

wherein:

R is hydrogen, a lower alkyl group, a lower alkynyl group, a lower trihaloalkyl group, a lower monohaloalkyl group, a lower alkenyl group, and aryl group, or an aralkyl group;

R^1 is hydrogen or methyl;

R^2 is methylene ($=CH_2$), cyano, hydroxymethyl, methoxymethylene ($=CHOCH_3$), acetyl, 2'-hydroxyacetyl, 2'-hydroxyacetyl acetate, 1-hydroxyethyl, 2'-hydroxyacetyl hemisuccinate, 2'-methoxyacetyl, pyrid-4-ylthioacetyl, ethylene ($=CHCH_3$), propylene ($=CHCH_2CH_3$), 2'-hydroxyacetyl hemisuccinate sodium salt, 1-hydroxybutyl, and 1-hydroxy-1-methylethyl, 1-hydroxypropyl, 1-propionyl, 3-methoxypropionyl, ethynyl, 2'-mesyloxyacetyl, or 1'-(ethylenedioxy)ethyl;

and pharmaceutically acceptable 3-esters, 20-esters, 21-esters, 3,20-diesters, and 3,21-diesters, thereof;

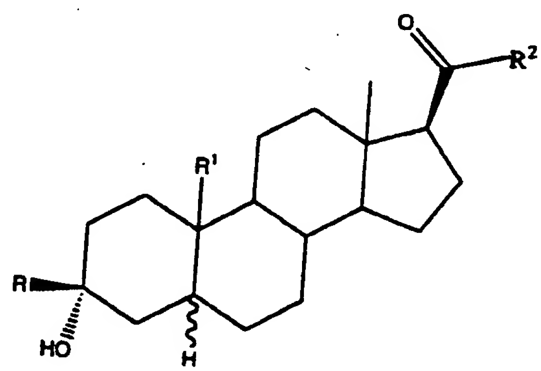
with the provisos that the steroid is not 3α,21-dihydroxy-5α-pregnan-20-one.

10. The method of claim 9 wherein the level of REM sleep is maintained compared to that found in normal sleep.

11. The method of claim 9 wherein substantial rebound insomnia is not induced.

12. The method of claim 9 wherein R is not hydrogen, the compound has an IC_{50} value of 5-300 nM in TBPS binding assays, and the compound is orally active.

13. The method of claim 9 wherein the compound has the structural formula II:



II

wherein:

R is a lower alkyl group, a lower alkynyl group, or a trihalo(lower) aklyl group;

R^1 is hydrogen or a lower alkyl group;

R^2 is hydrogen, hydroxy, a pyrid-4-ylthio group, a hemisuccinoyloxy group, or a phosphoryloxy group or the sodium salts thereof; with the provisos that:

(1) when R is hydrogen, R^2 is a pyrid-4-ylthio group;

(2) when R^1 is hydrogen, R is a trihalo(lower) alkyl group; and

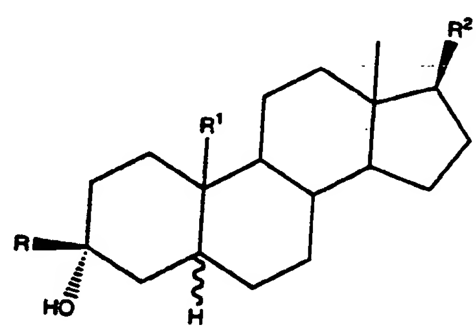
(3) when R^2 is other than hydrogen or a pyrid-4-ylthio group, R is a trihalo(lower) alkyl group.

14. The method of claim 13 wherein R is selected from the group consisting of lower alkynyl, and trihalo (lower) alkyl.

15. The method of claim 13 wherein R^2 selected from the group consisting of hemisuccinoyloxy, and phosphoryloxy and their sodium salts.

16. The method of claim 13 wherein the compound is selected from the group consisting of 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, 3α-hydroxy-3β-methyl-5α-pregnan-20-one, 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate sodium salt, 3α-hydroxy-3β-ethynyl-5α-pregnan-20-one, 3α,21-dihyrdoxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate, 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one, 3α-hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one, and 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one 21-phosphate disodium salt.

17. A method of treating insomnia comprising administering to an animal subject an amount of a 3α-hydroxy-5-reduced steroid or derivative thereof that will increase the duration of uninterrupted NREM sleep, and will maintain the level of motor function and motivation found in normal sleep, said steroid having the structural formula I:



I

wherein:

R is hydrogen, a lower alkyl group, a lower alkynyl group, a lower trihaloalkyl group, a lower monohaloalkyl

group, a lower alkenyl group, and aryl group, or an aralkyl group;

R^1 is hydrogen or methyl;

R^2 is methylene (=CH_2), cyano, hydroxymethyl, methoxymethylene (=$CHOCH_3$), acetyl, 2'-hydroxyacetyl, 2'-hydroxyacetyl acetate, 1-hydroxyethyl, 2'-hydroxyacetyl hemisuccinate, 2'-methoxyacetyl, pyrid-4-ylthioacetyl, ethylene (=$CHCH_3$), propylene (=$CHCH_2CH_3$), 2'-hydroxyacetyl hemisuccinate sodium salt, 1-hydroxybutyl, and 1-hydroxy-1-methylethyl, 1-hydroxypropyl, 1-propionyl, 3-methoxypropionyl, ethynyl, 2'-mesyloxyacetyl, or 1'-(ethylenedioxy)ethyl;

and pharmaceutically acceptable 3-esters, 20-esters, 21-esters, 3,20-diesters, and 3,21-diesters, thereof;

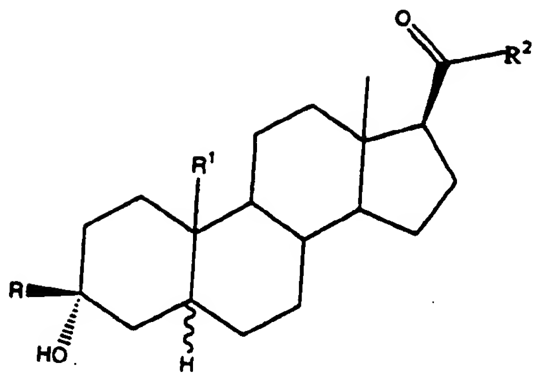
with the provisos that the steroid is not 3α,21-dihydroxy-5α-pregnan-20-one.

18. The method of claim 17 wherein the level of REM sleep is maintained compared to that found in normal sleep.

19. The method of claim 17 wherein substantial rebound insomnia is not induced.

20. The method of claim 17 wherein R is not hydrogen, the compound has an IC_{50} value of 5-300 nM in TBPS binding assays, and the compound is orally active.

21. The method of claim 17 wherein the compound has the structural formula II:



II

wherein:

R is a lower alkyl group, a lower alkynyl group, or a trihalo(lower) alkyl group;

R^1 is hydrogen or a lower alkyl group;

R^2 is hydrogen, hydroxy, a pyrid-4-ylthio group, a hemisuccinoyloxy group, or a phosphoryloxy group or the sodium salts thereof; with the provisos that:

(1) when R is hydrogen, R^2 is a pyrid-4-ylthio group;

(2) when R^1 is hydrogen, R is a trihalo(lower) alkyl group; and

(3) when R^2 is other than hydrogen or a pyrid-4-ylthio group, R is a trihalo(lower) alkyl group.

22. The method of claim 21 wherein R is selected from the group consisting of lower alkynyl, and trihalo (lower) alkyl.

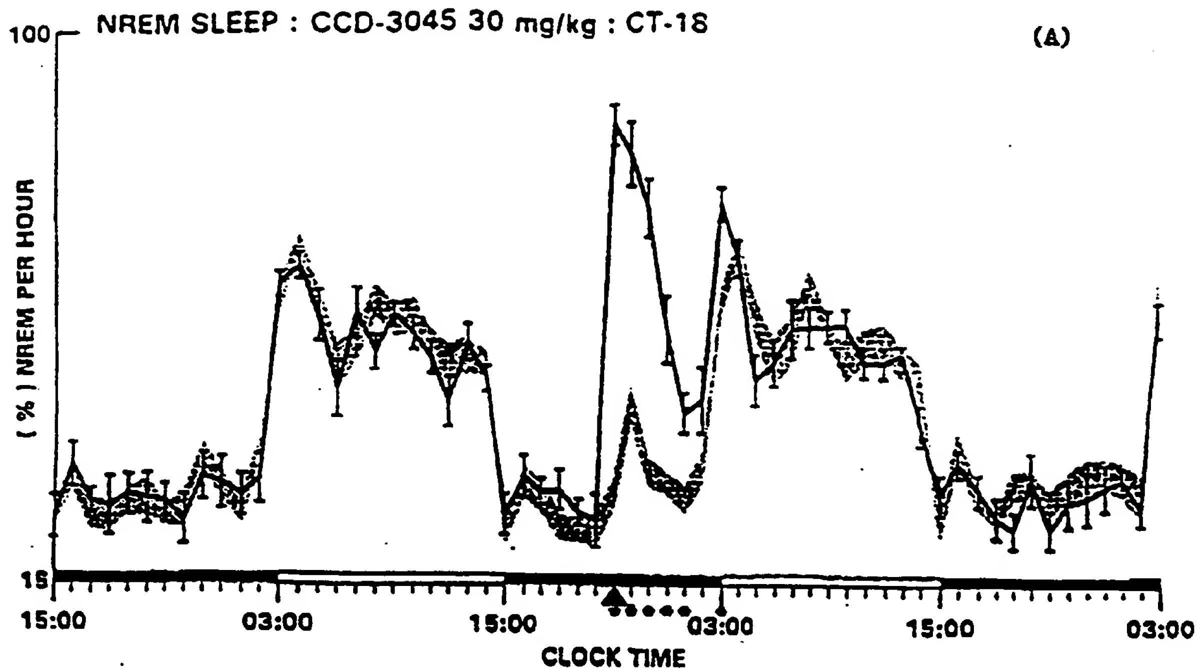
23. The method of claim 21 wherein R^2 selected from the group consisting of hemisuccinoyloxy and phosphoryloxy and their sodium salts.

24. The method of claim 21 wherein the compound is selected from the group consisting of 3α-hydroxy-3β-trifluoromethyl-5β-pregnan-20-one 21-phosphate disodium salt, 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one, 3α-hydroxy-3β-methyl-5α-pregnan-20-one, 3α,21-dihydroxy-3β-

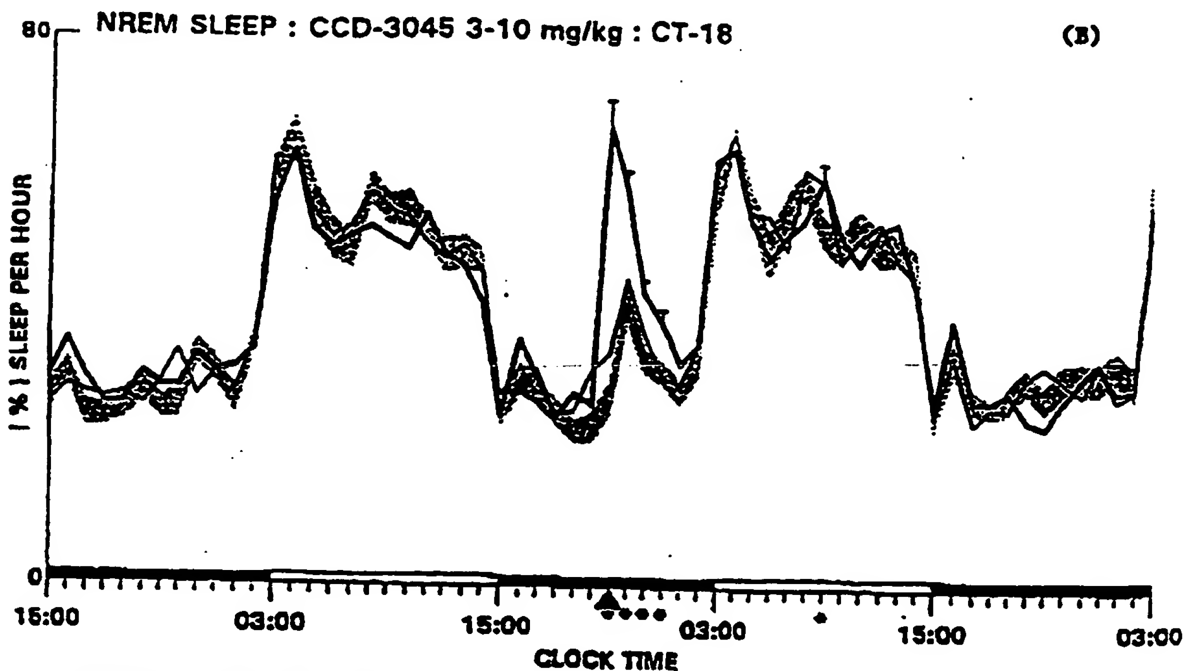
trifluoromethyl-5β-pregnan-20-one 21-hemisuccinate sodium salt, 3α-hydroxy-3β-ethynyl-5α-pregnan-20-one, 3α,21-dihyrdoxy-3β-ethynyl-5β-pregnan-20-one 21-hemisuccinate, 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one,3α-hydroxy-21-(pyrid-4-ylthio)-5β-pregnan-20-one, and 3α-hydroxy-3β-trifluoromethyl-5β-19-nor-pregnan-20-one 21-phosphate disodium salt.

25. A method for treating insomnia comprising administering orally to a patient in need of such treatment an amount of 3α-hydroxy-5β-pregnan-20-one effective to induce sleep without inducing anesthesia.

26. A method of inducing sleep without hypnosis comprising administering orally to a patient a pharmaceutically effective amount of 3α-hydroxy-5β-pregnan-20-one.



Shaded area (N = 15) : methylcellulose vehicle
Heavy line (N = 12) : CCD-3045 30 mg/kg



Shaded area (N = 20) : methylcellulose vehicle
Heavy line (N = 13) : CCD-3045 10 mg/kg
Light line (N = 12) : CCD-3045 3 mg/kg

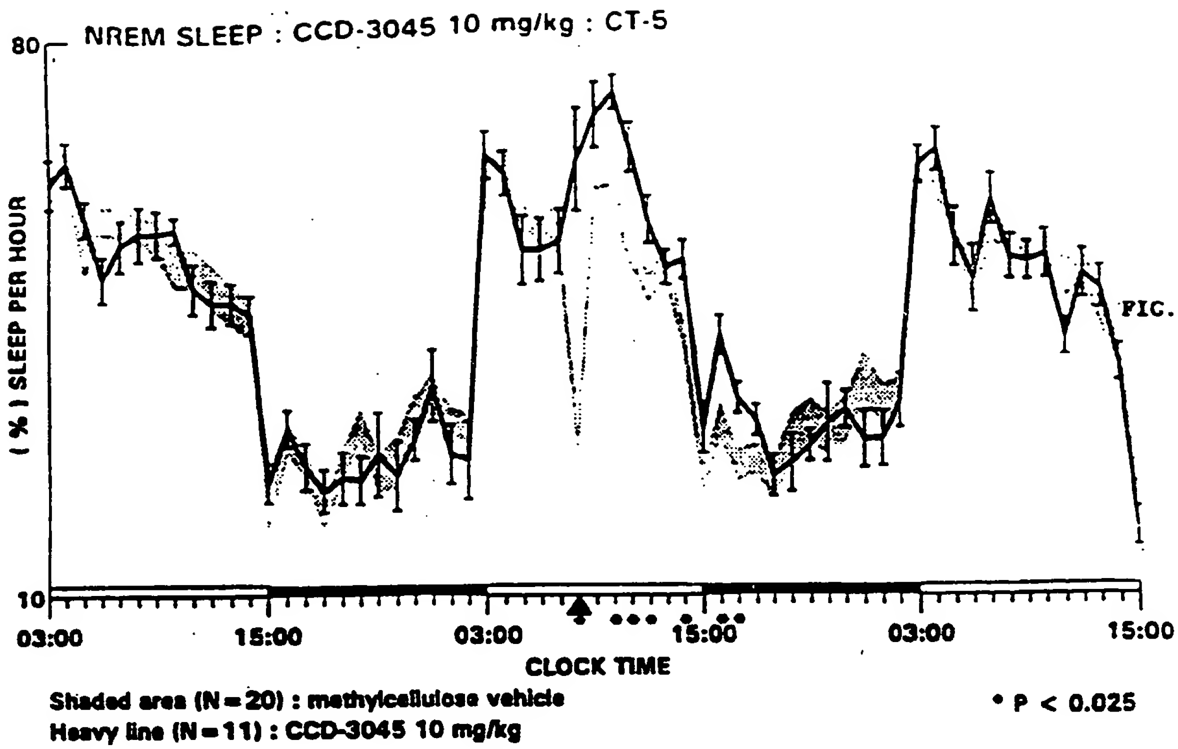
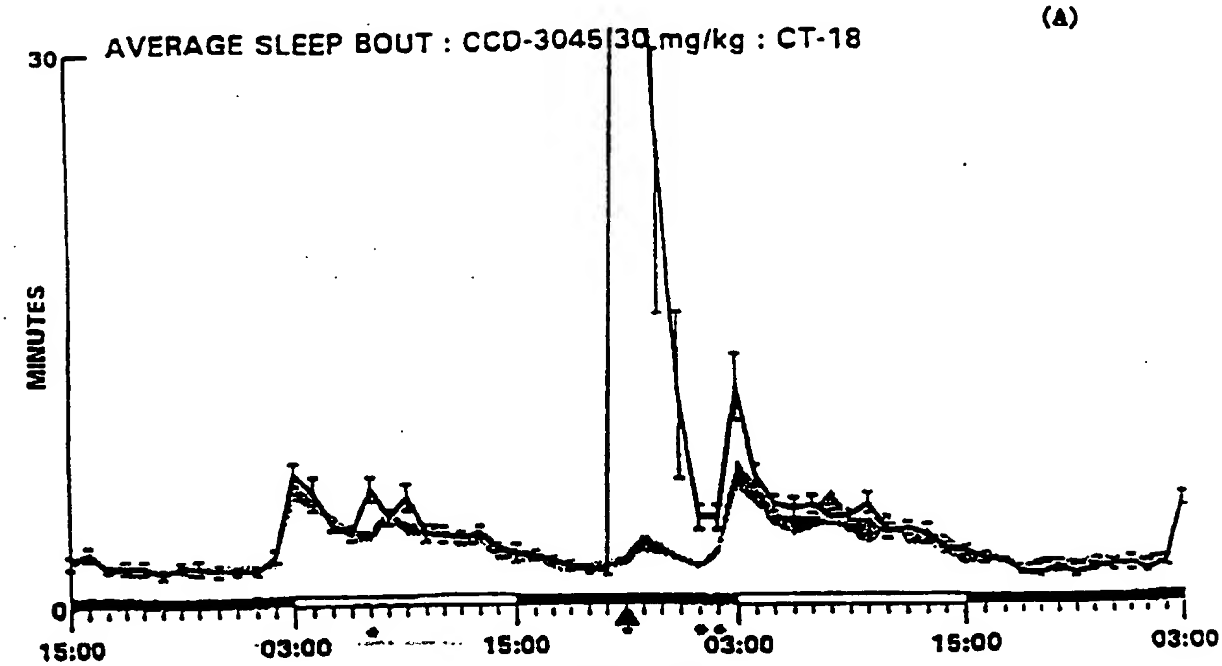
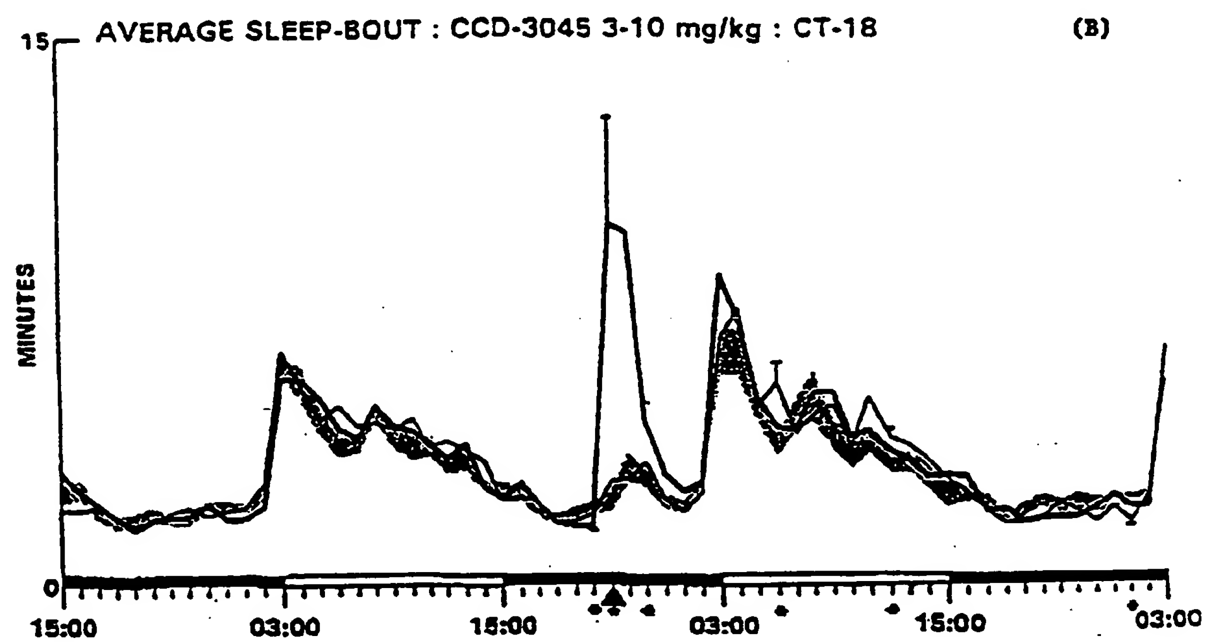

NREM SLEEP : CCD-3045 10 mg/kg : CT-5
80
10
(%) SLEEP PER HOUR
03:00
15:00
03:00
15:00
03:00
15:00
CLOCK TIME
Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=11) : CCD-3045 10 mg/kg
* P < 0.025

FIG. 1C

FIGURE II



Shaded area (N=15) : methylcellulose vehicle
Heavy line (N=12) : CCD-3045 30 mg/kg



Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=13) : CCD-3045 10 mg/kg
Light line (N=12) : CCD-3045 3 mg/kg

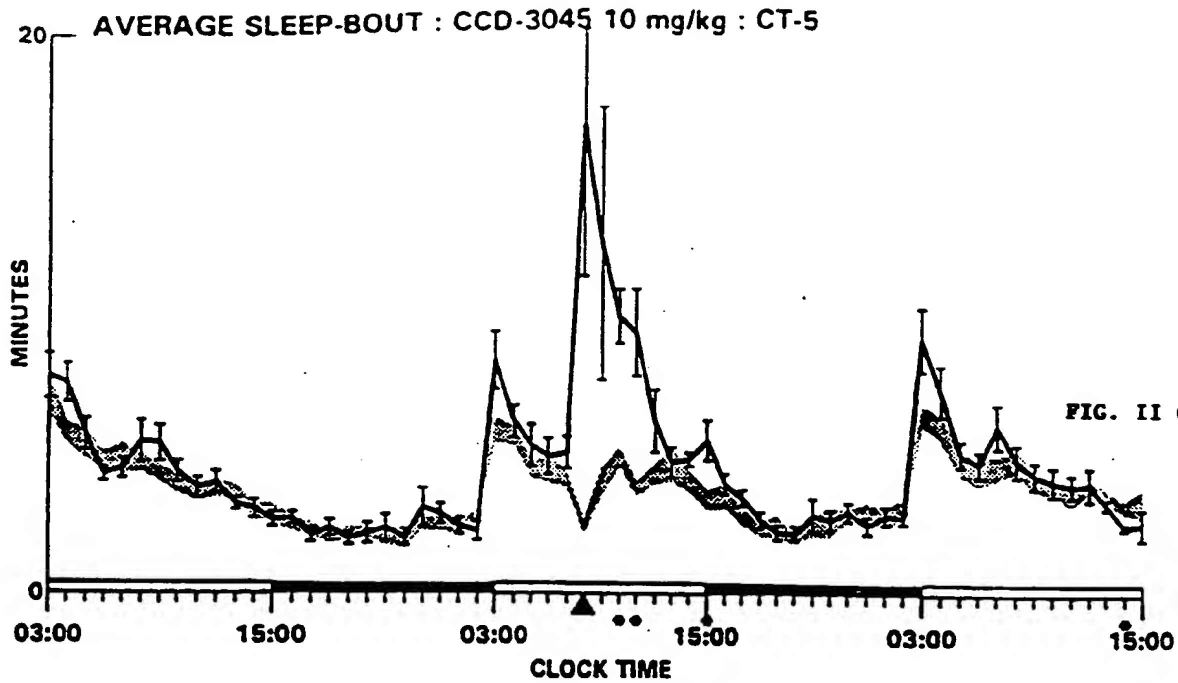
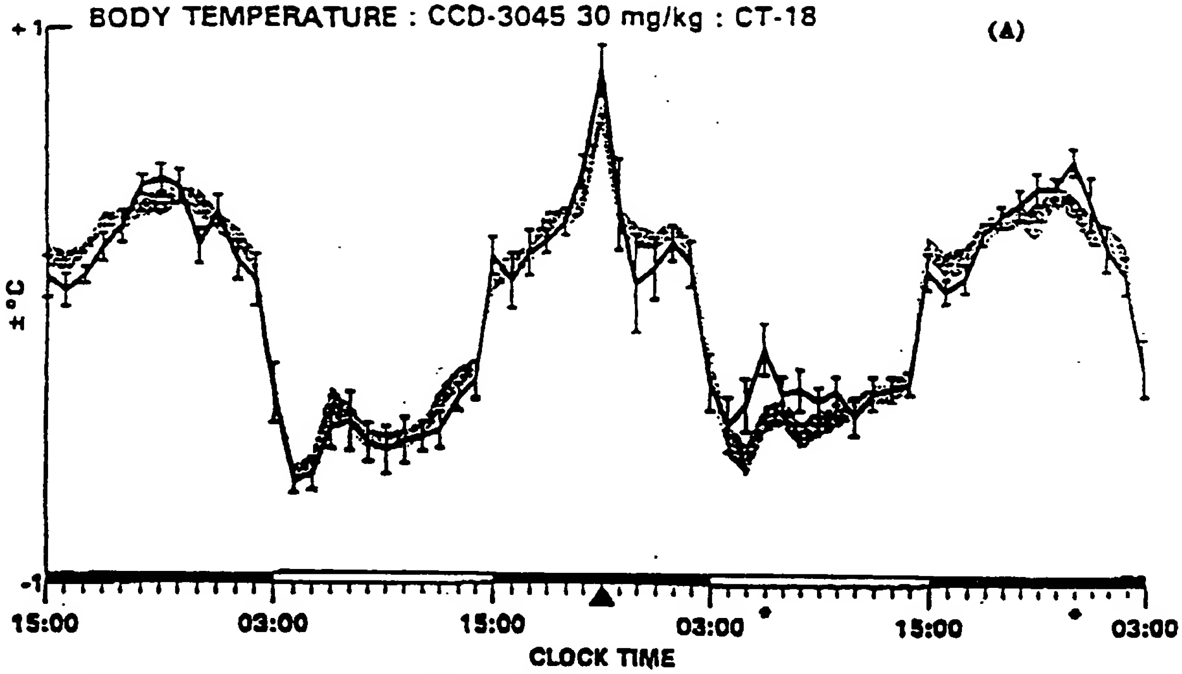

AVERAGE SLEEP-BOUT : CCD-3045 10 mg/kg : CT-5
20
0
MINUTES
03:00
15:00
03:00
15:00
03:00
15:00
CLOCK TIME

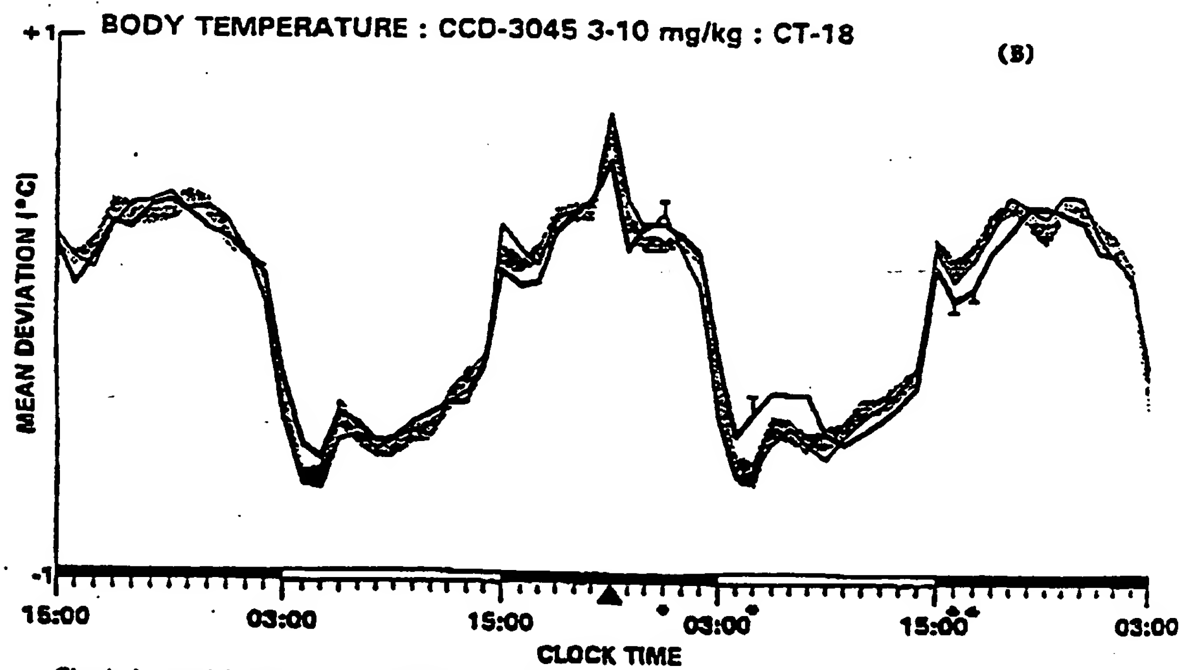
FIG. II C

FIGURE III



Shaded area (N=15) : methylcellulose vehicle
Heavy line (N=8) : CCD-3045 30 mg/kg

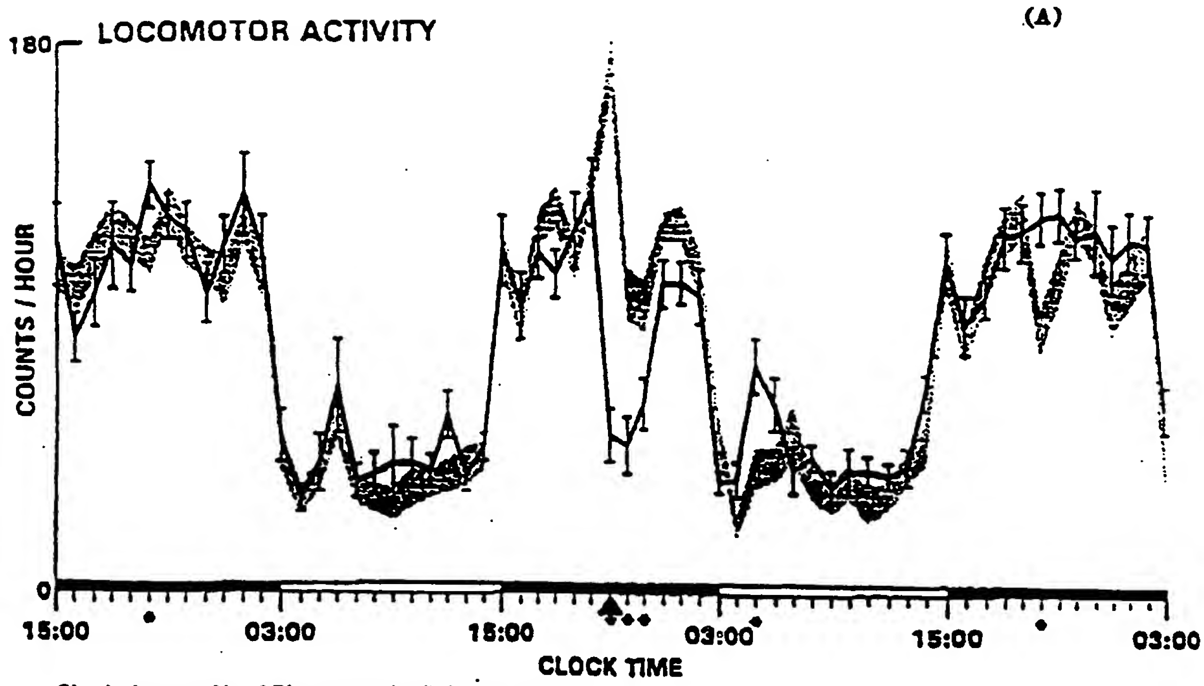
* P < 0.025



Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=10) : CCD-3045 10 mg/kg
Light line (N=12) : CCD-3045 3 mg/kg

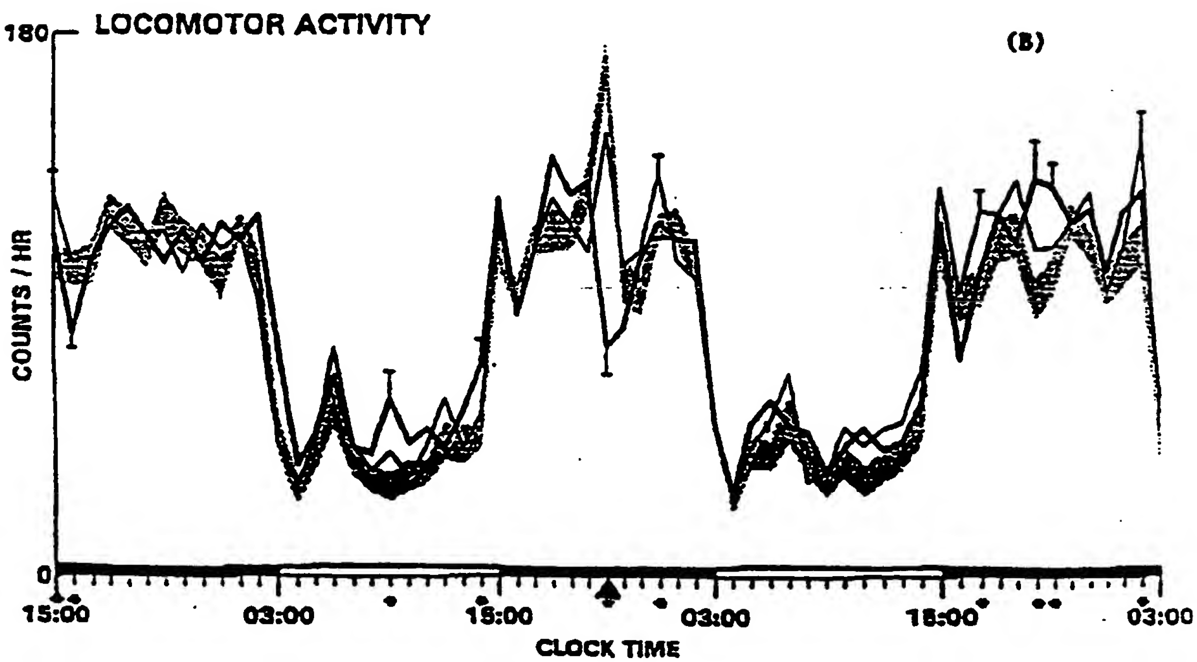
* P < 0.025
(vs Shaded area)

FIGURE IV



Shaded area (N=15) : methylcellulose vehicle
Heavy line (N=12) : CCD-3045 30 mg/kg

* P < 0.025



Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=12) : CCD-3045 10 mg/kg
Light line (N=13) : CCD-3045 3 mg/kg

* P < 0.025
(vs Shaded area)

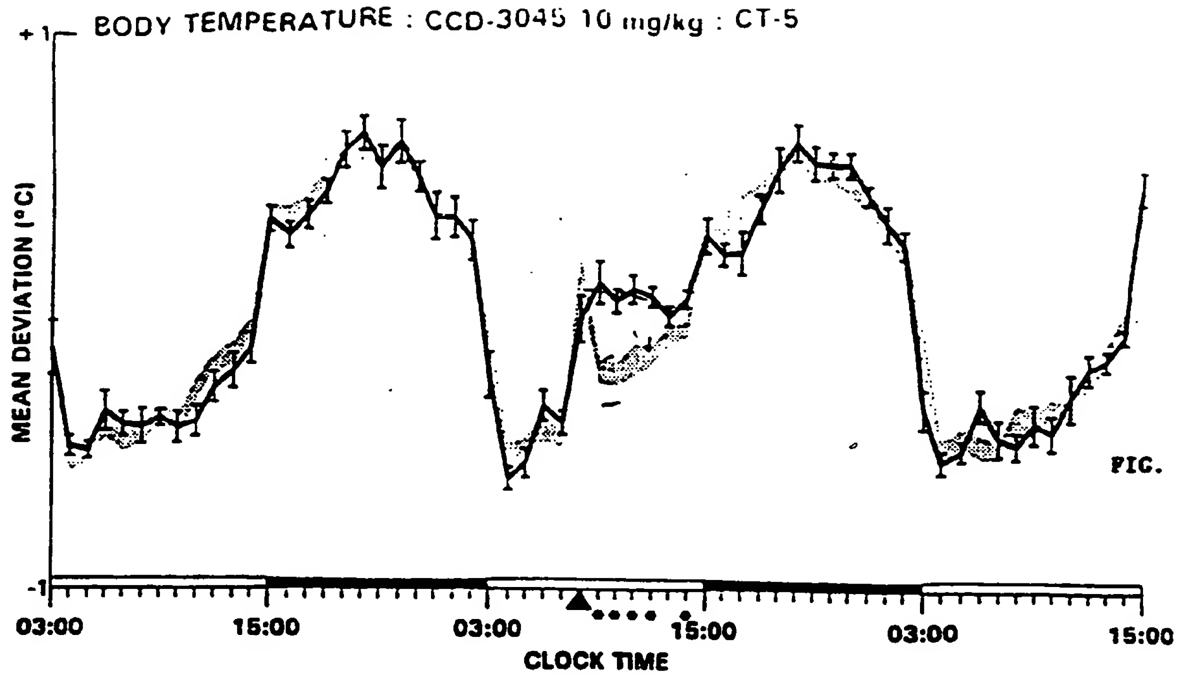


FIG. III C

Shaded area (N=20) : vehicle
Heavy line (N=10) : CCD-3045 10 mg/kg

* P < 0.025

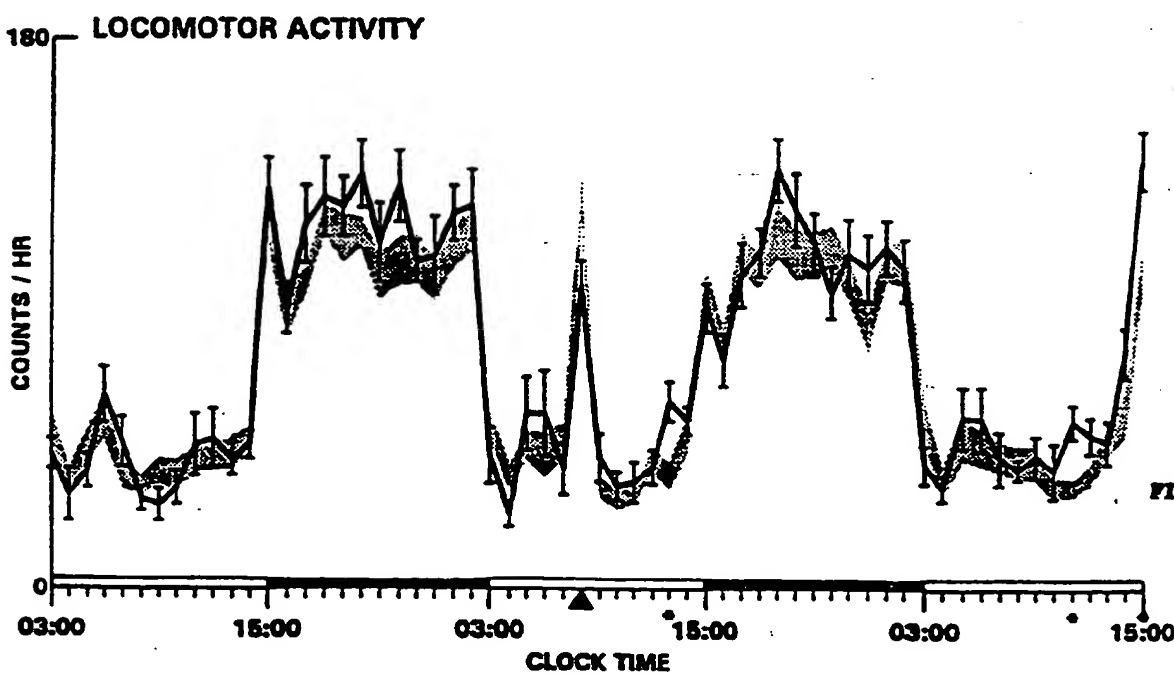
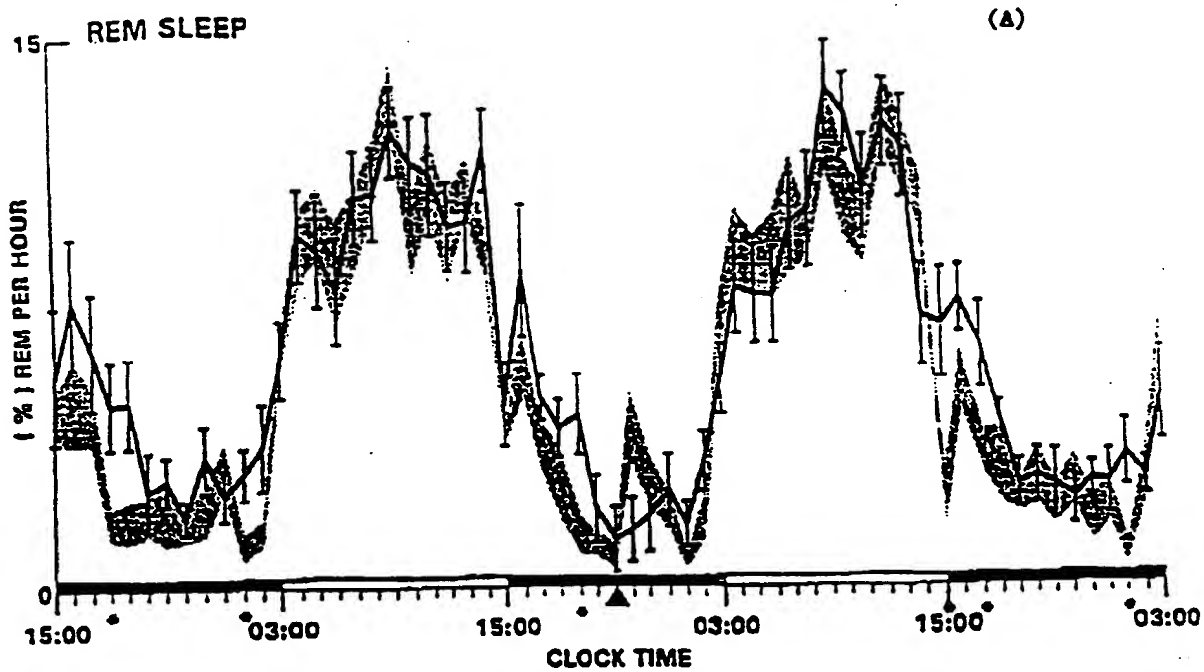


FIG. IV C

Shaded area (N=20) : vehicle
Heavy line (N=11) : CCD-3045 10 mg/kg

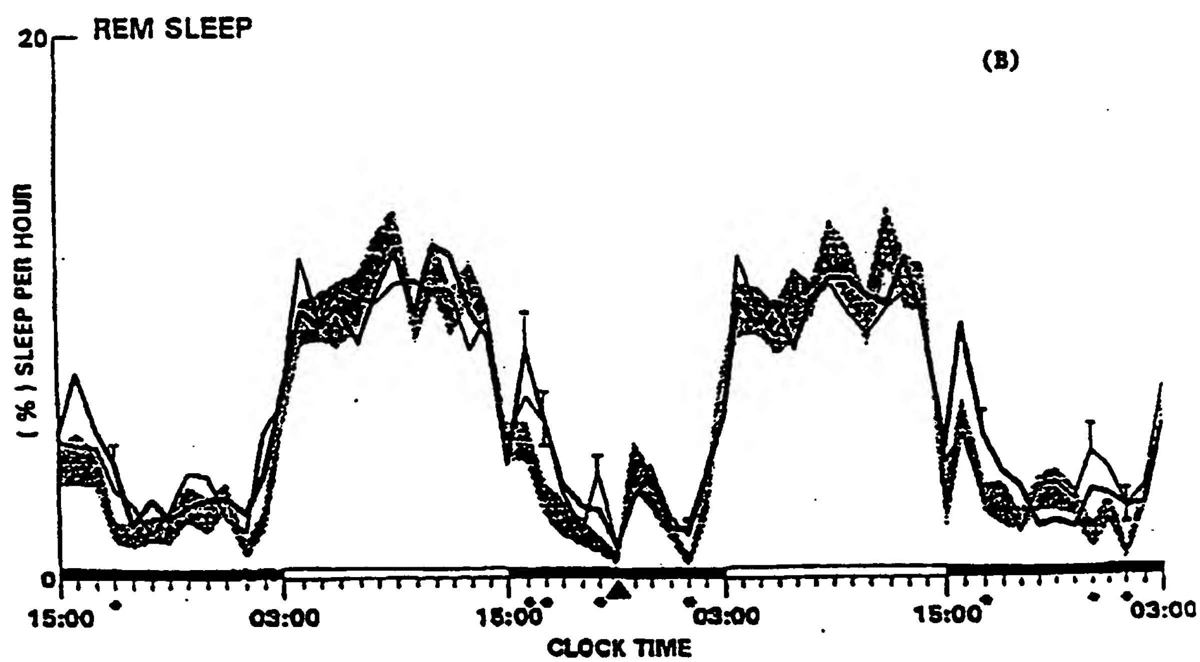
* P < 0.025

FIGURE V



Shaded area (N = 15) : methylcellulose vehicle
Heavy line (N = 12) : CCD-3045 30 mg/kg

• P < 0.025



Shaded area (N = 20) : methylcellulose vehicle
Heavy line (N = 13) : CCD-3045 10 mg/kg
Light line (N = 12) : CCD-3045 3 mg/kg

• P < 0.025
(vs Shaded area)

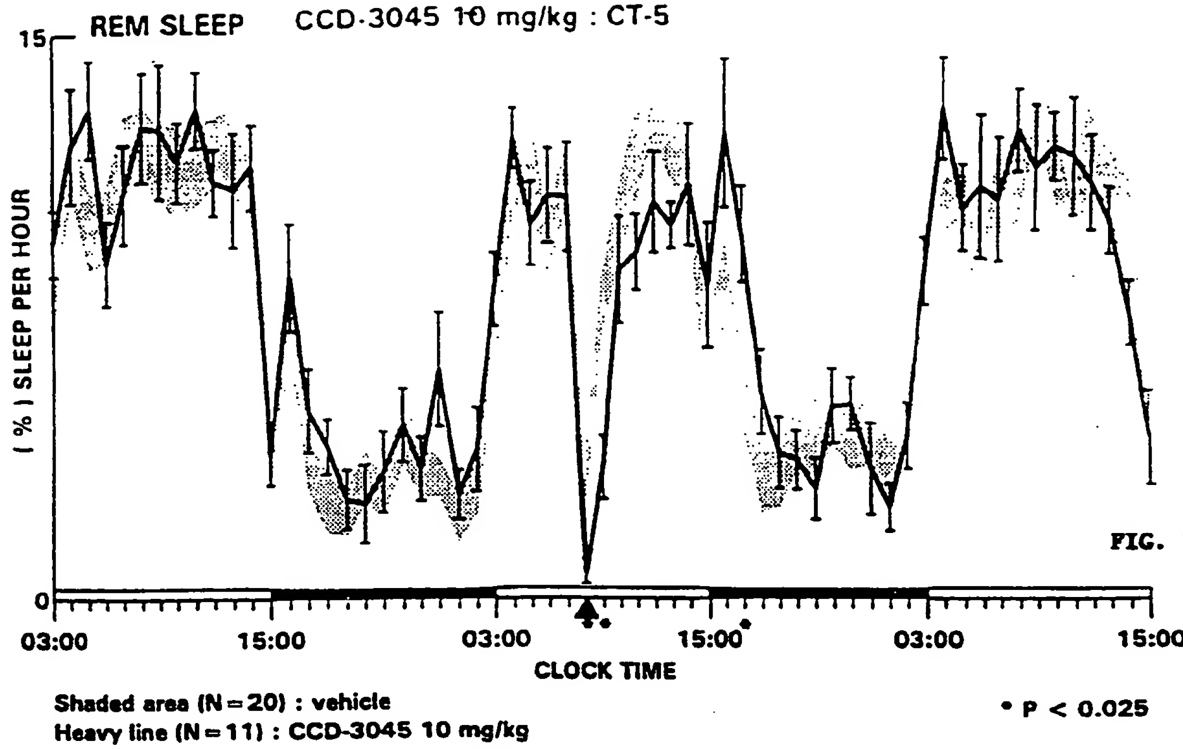

REM SLEEP
CCD-3045 10 mg/kg : CT-5
15
0
(%) SLEEP PER HOUR
03:00
15:00
03:00
15:00
03:00
15:00
CLOCK TIME
Shaded area (N=20) : vehicle
Heavy line (N=11) : CCD-3045 10 mg/kg
* P < 0.025

FIG. V C

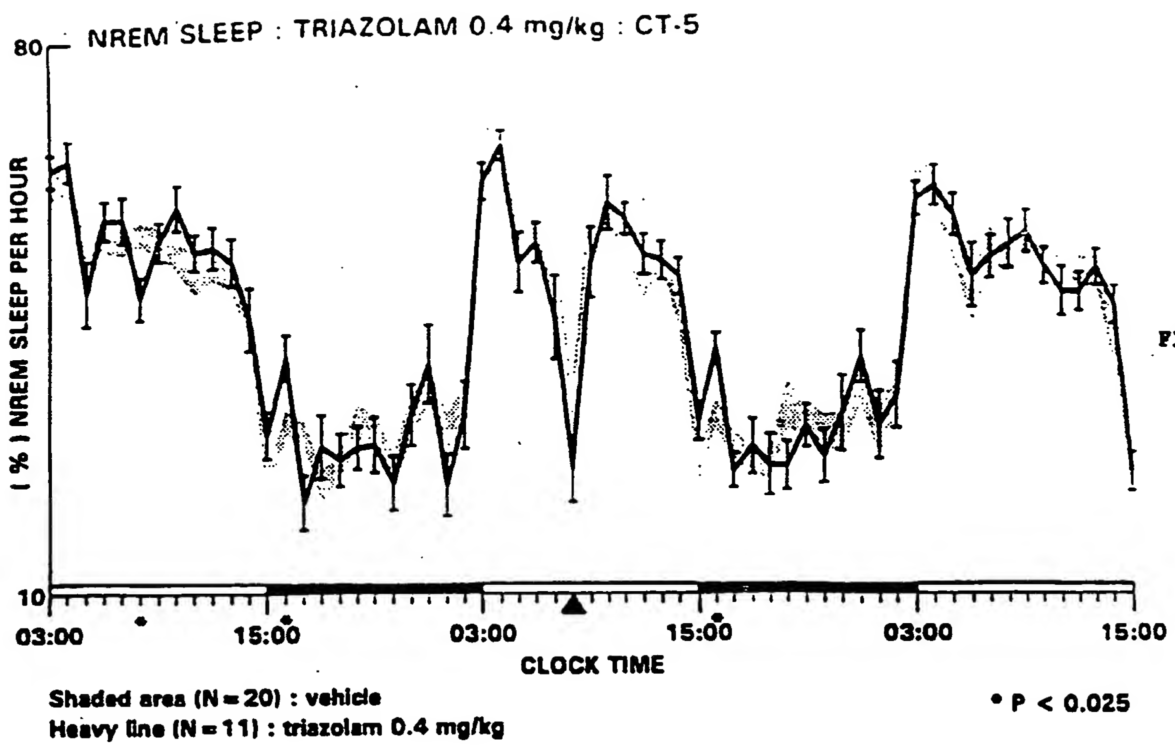


FIG. VI A

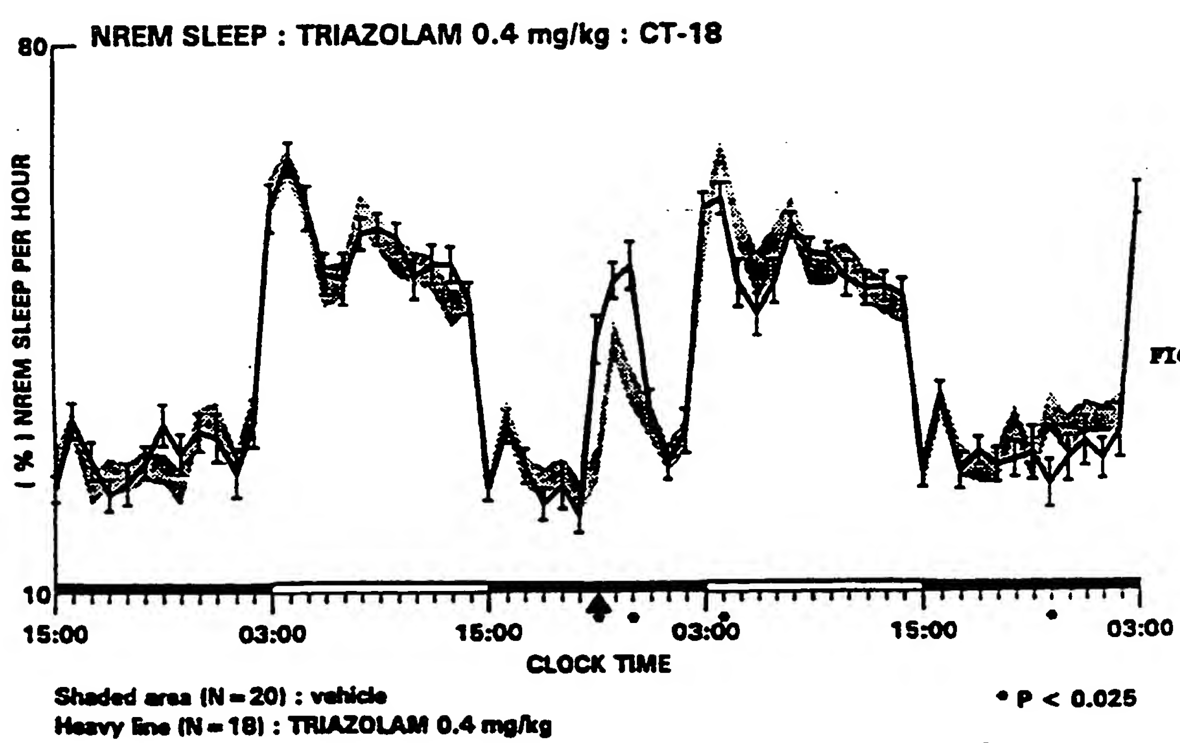
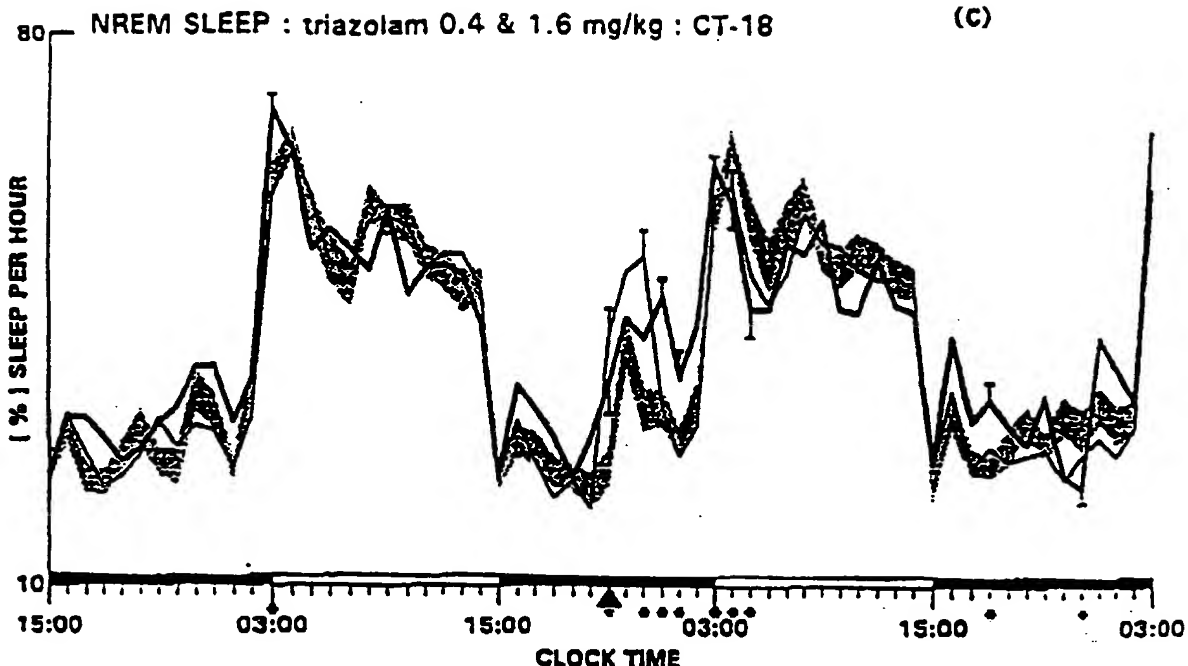


FIG. VI B

FIGURE VI



Shaded area (N = 15) : methylcellulose vehicle
Heavy line (N = 12) : CCD-3045 30 mg/kg

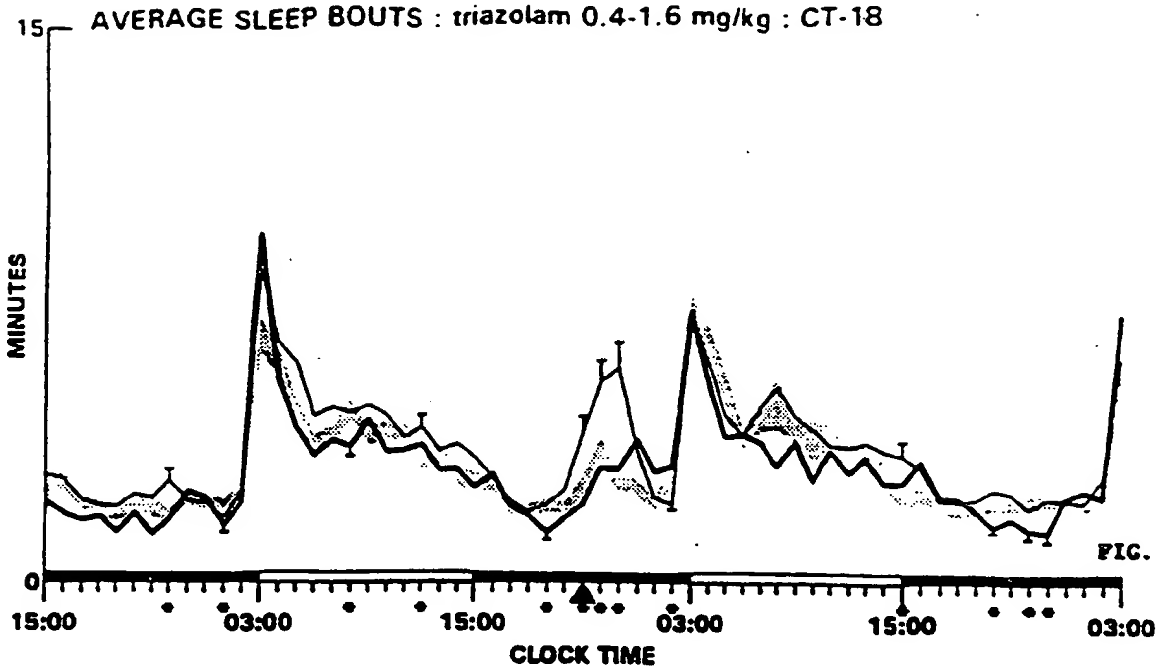

AVERAGE SLEEP BOUTS : triazolam 0.4-1.6 mg/kg : CT-18
15
0
MINUTES
15:00
03:00
15:00
03:00
15:00
03:00
CLOCK TIME

FIG. VI D

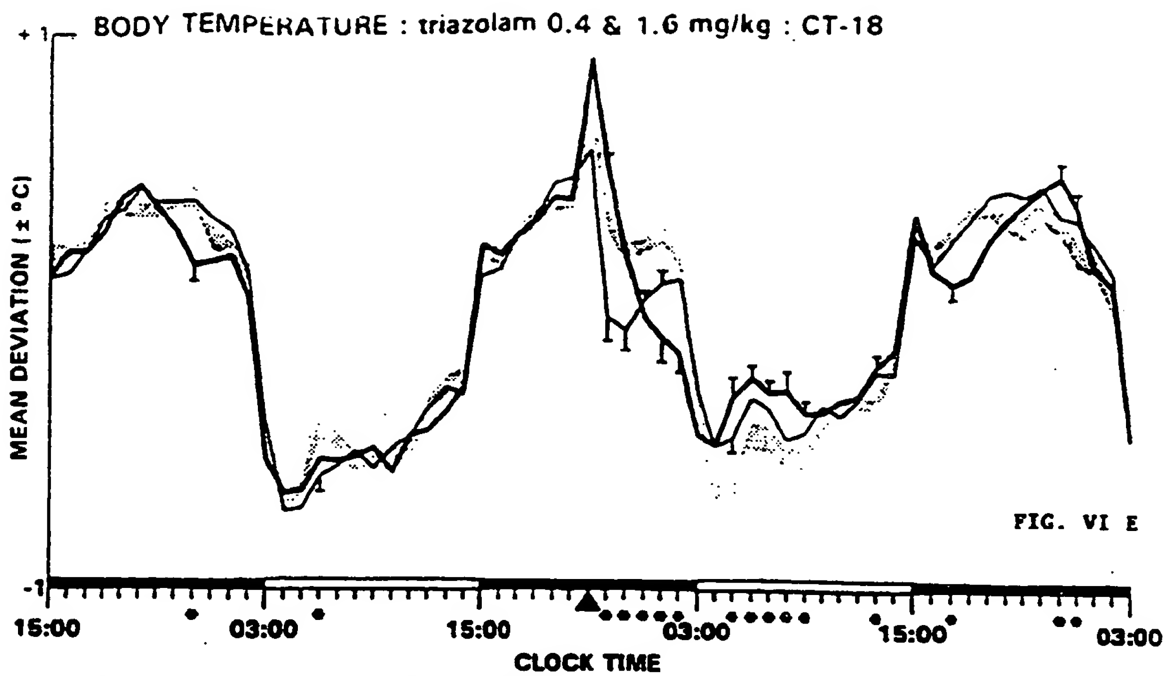


FIG. VI E

Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=11) : TRZ 1.6 mg/kg
Light line (N=15) : TRZ 0.4 mg/kg

* P < 0.025
(vs Shaded area)

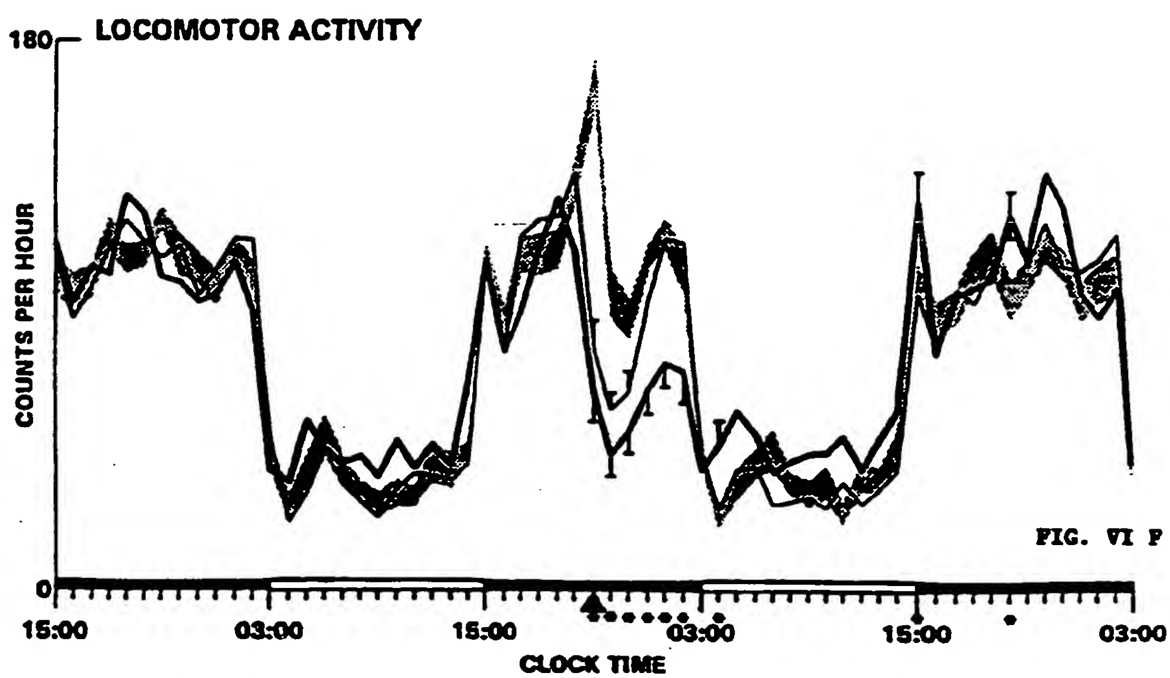


FIG. VI F

Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=12) : TRZ 1.6 mg/kg
Light line (N=17) : TRZ 0.4 mg/kg

* P < 0.025
(vs Shaded area)

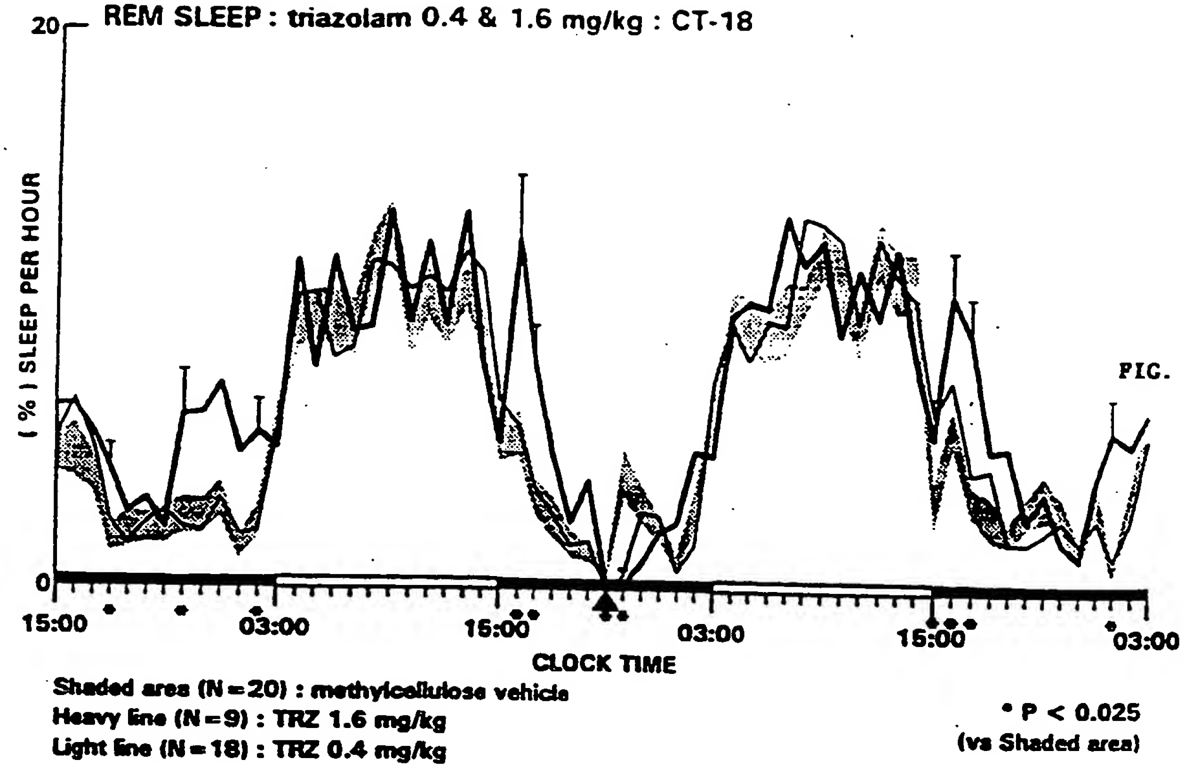
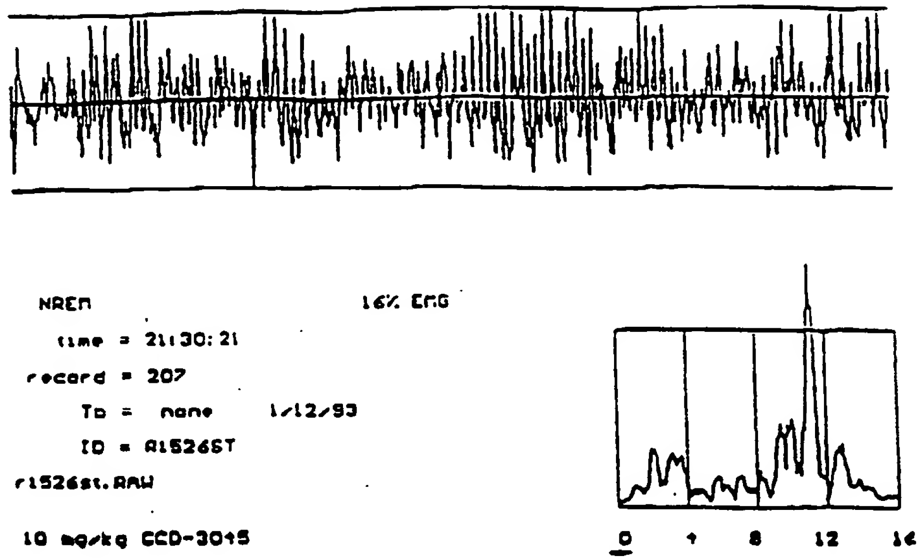

REM SLEEP : triazolam 0.4 & 1.6 mg/kg : CT-18
20
0
(%) SLEEP PER HOUR
15:00
03:00
15:00
03:00
15:00
03:00
CLOCK TIME
Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=9) : TRZ 1.6 mg/kg
Light line (N=18) : TRZ 0.4 mg/kg
* P < 0.025
(vs Shaded area)

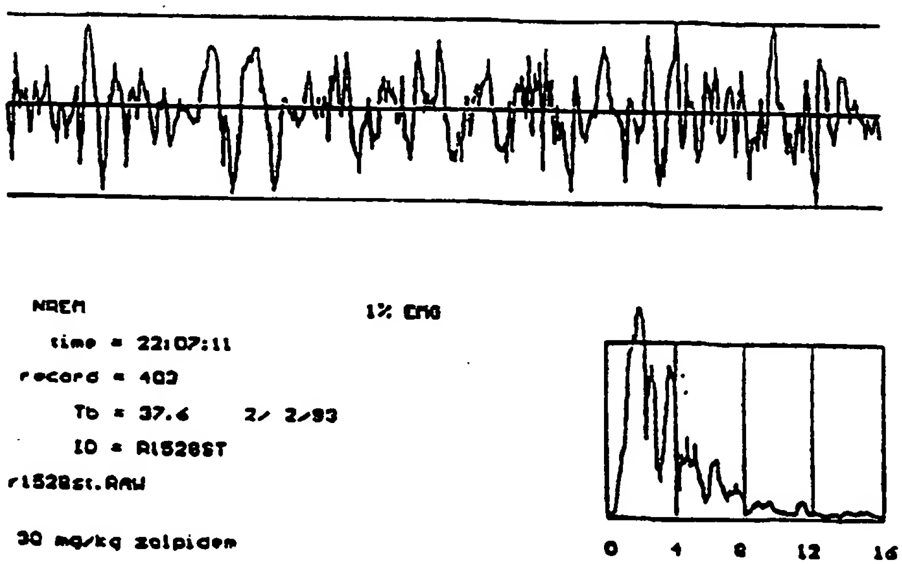
FIG. VI G

FIGURE VII

(A)



(B)



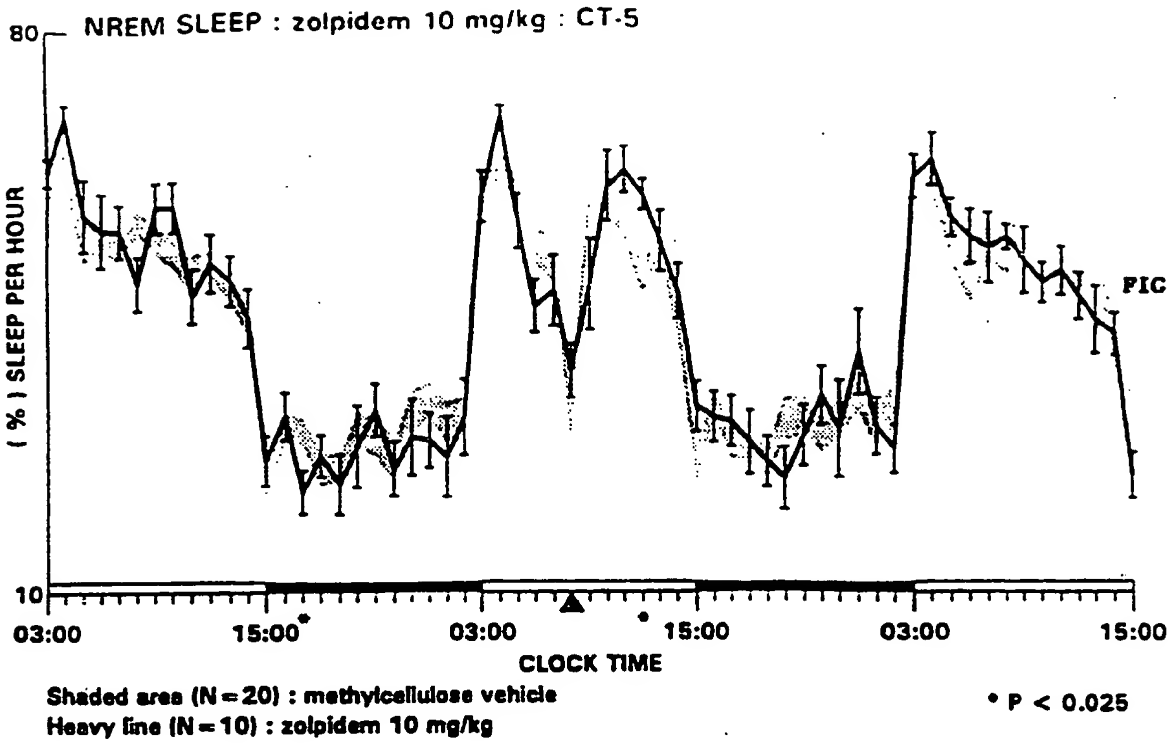


FIG. VIII A

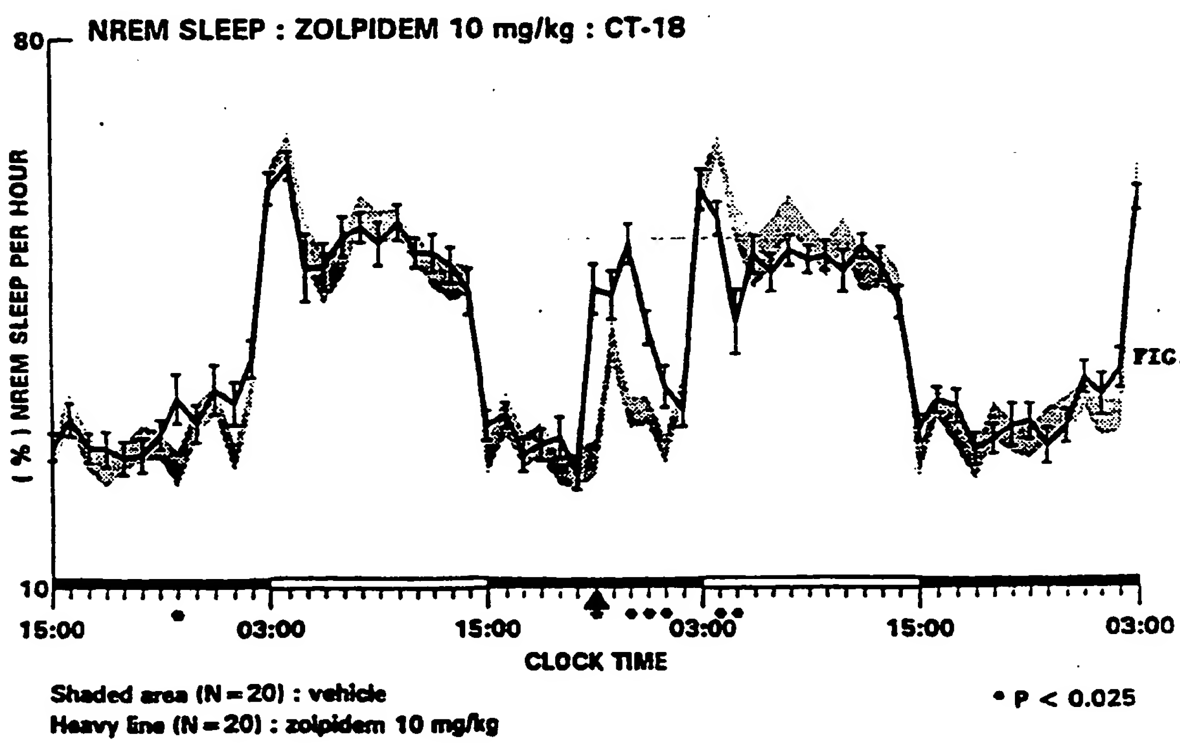
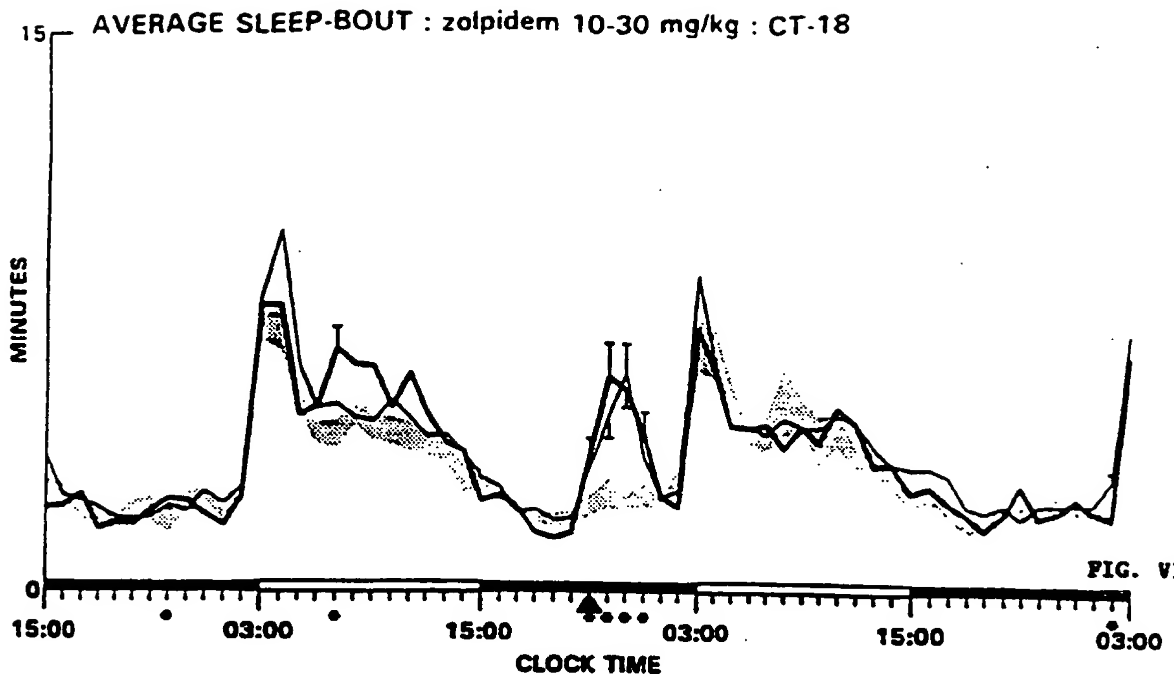


FIG. VIII B



AVERAGE SLEEP-BOUT : zolpidem 10-30 mg/kg : CT-18
15
0
MINUTES
15:00
03:00
15:00
03:00
15:00
03:00
CLOCK TIME

FIG. VIII C

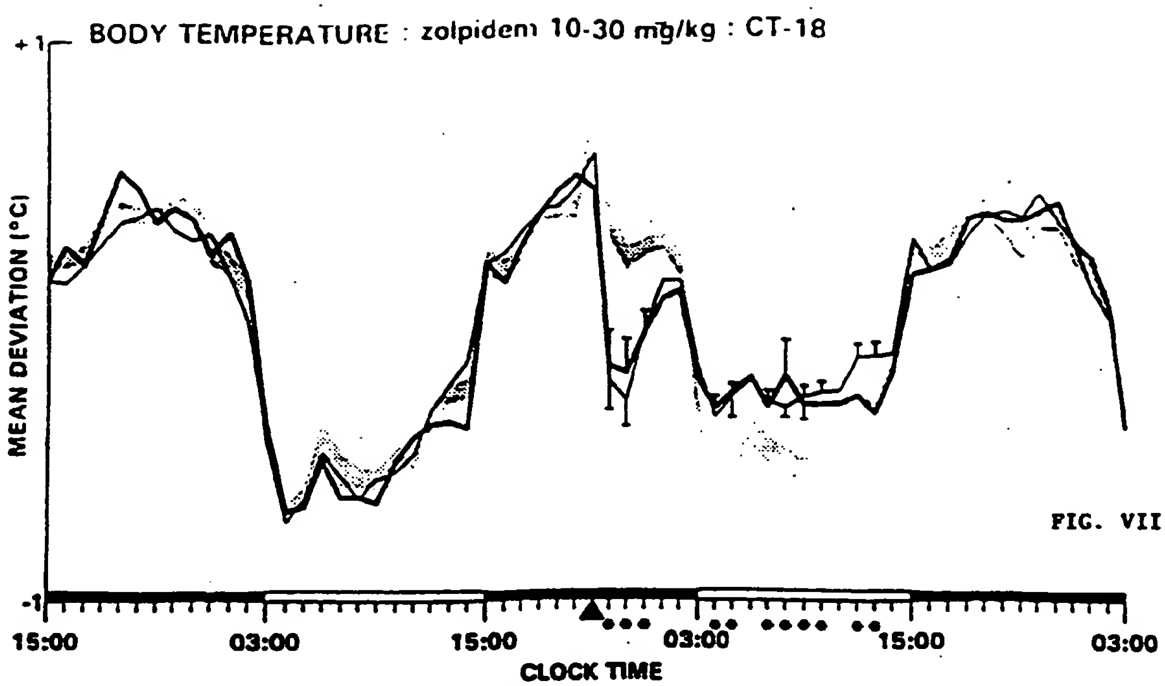


FIG. VIII D

Shaded area (N = 15) : methylcellulose vehicle
Heavy line (N = 6) : zolpidem 30 mg/kg
Light line (N = 18) : zolpidem 10 mg/kg

• P < 0.01
(vs Shaded area)

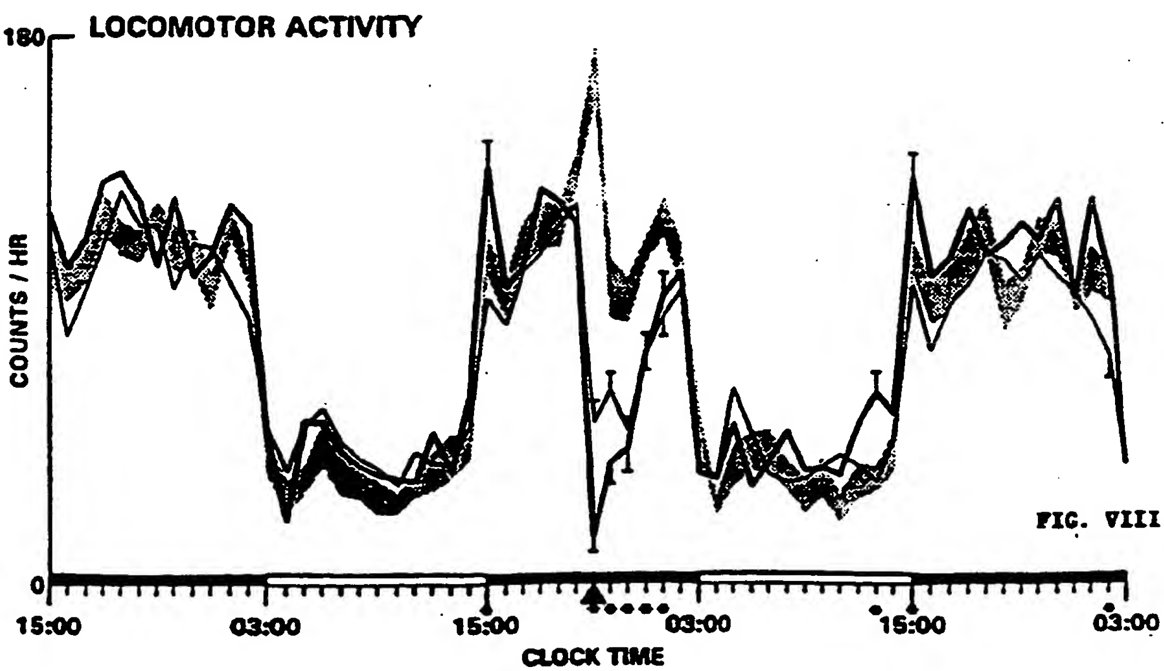


FIG. VIII E

Shaded area (N = 15) : methylcellulose vehicle
Heavy line (N = 7) : zolpidem 30 mg/kg
Light line (N = 19) : zolpidem 10 mg/kg

• P < 0.025
(vs Shaded area)

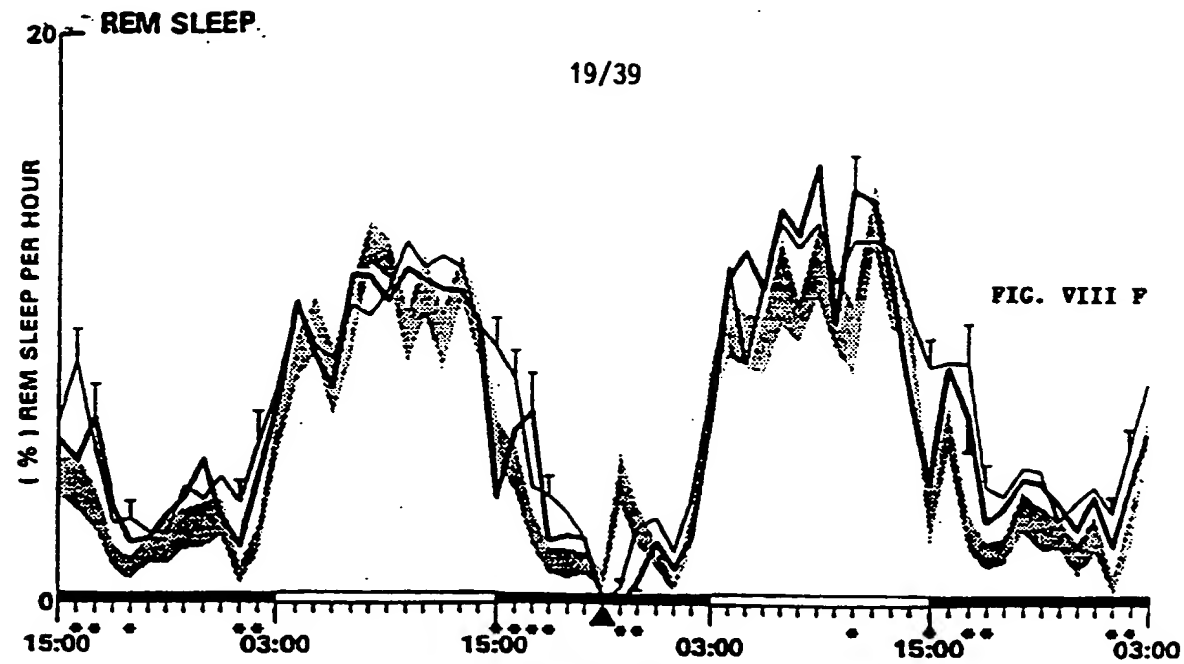


FIG. VIII F

Shaded area (N=15) : methylcellulose vehicle
Heavy line (N=9) : zolpidem 30 mg/kg
Light line (N=20) : zolpidem 10 mg/kg

* P < 0.025
(vs Shaded area)

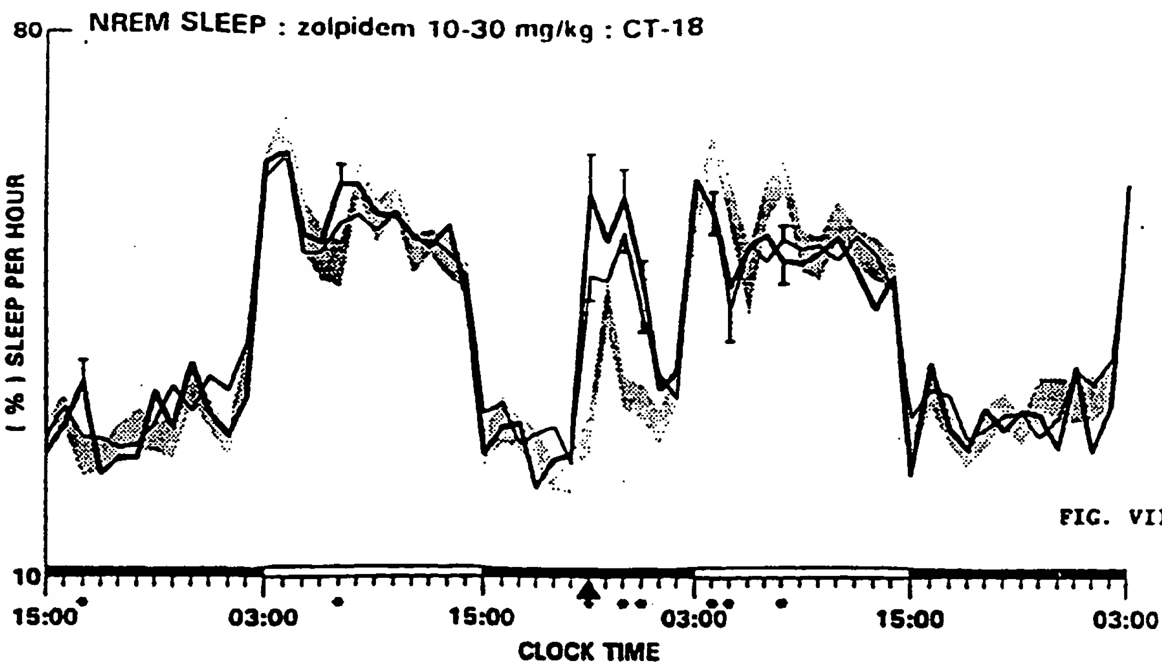


FIG. VIIIG

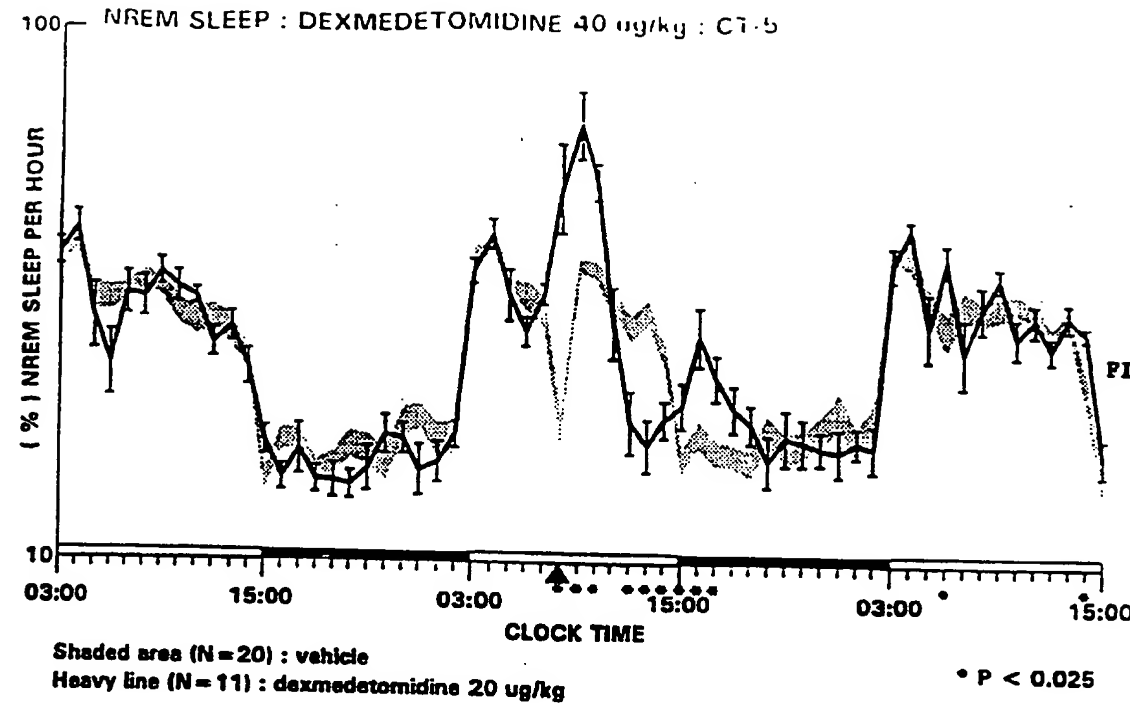


FIG. IX A

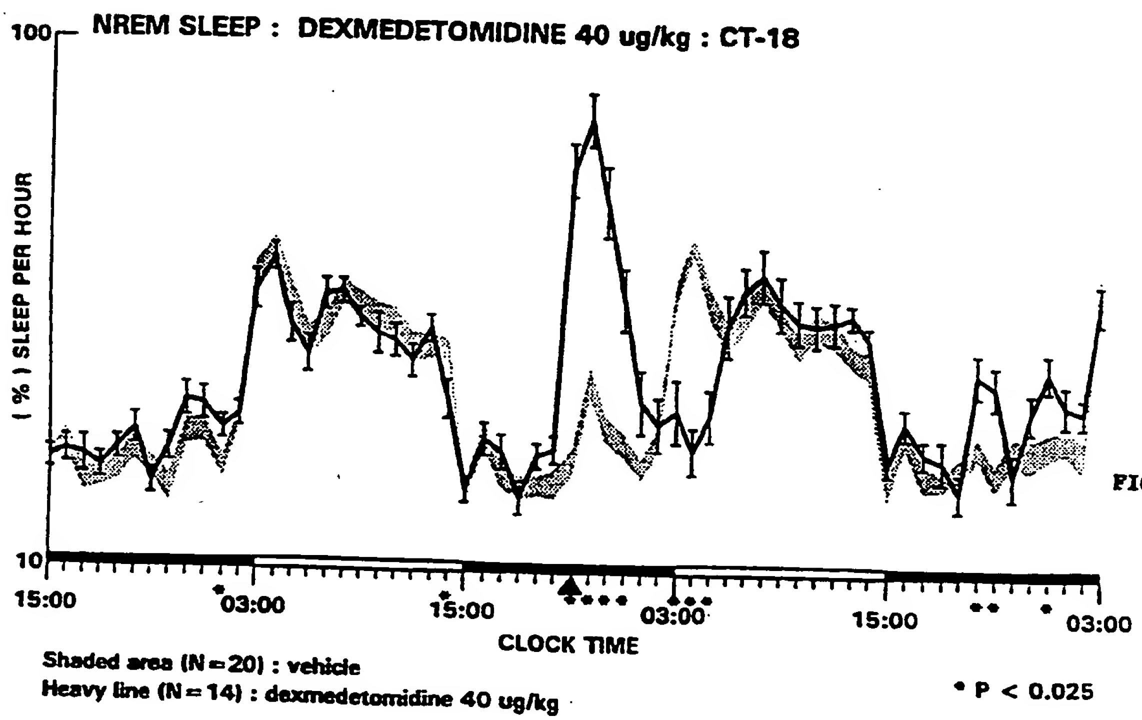


FIG. IX B

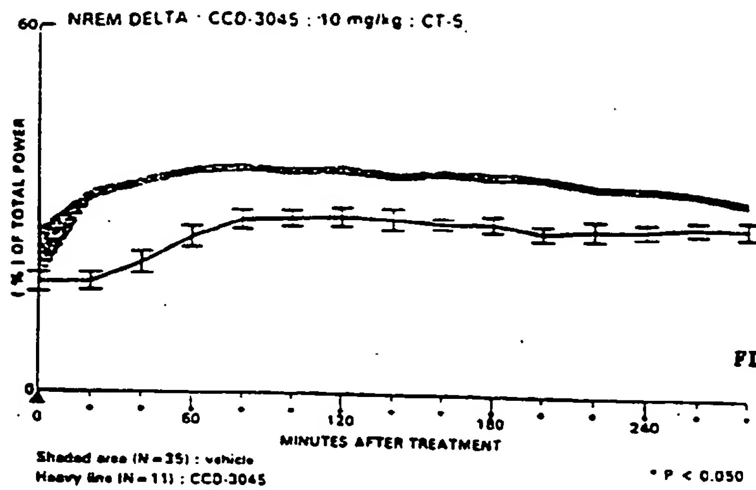


FIG. X A

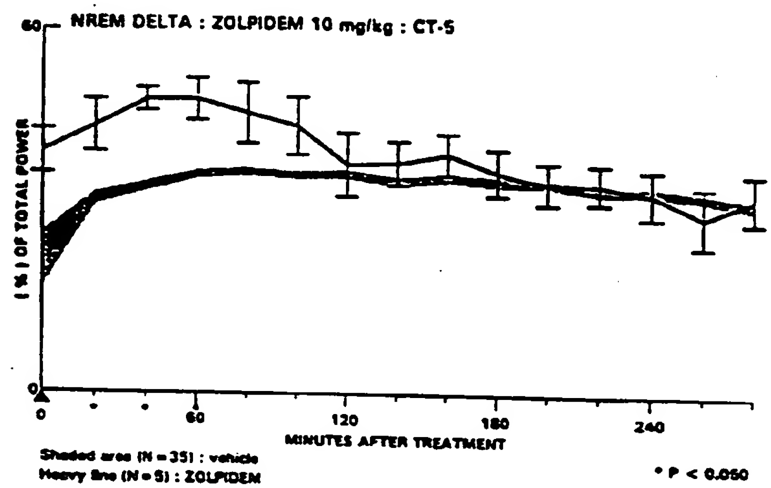


FIG. X B

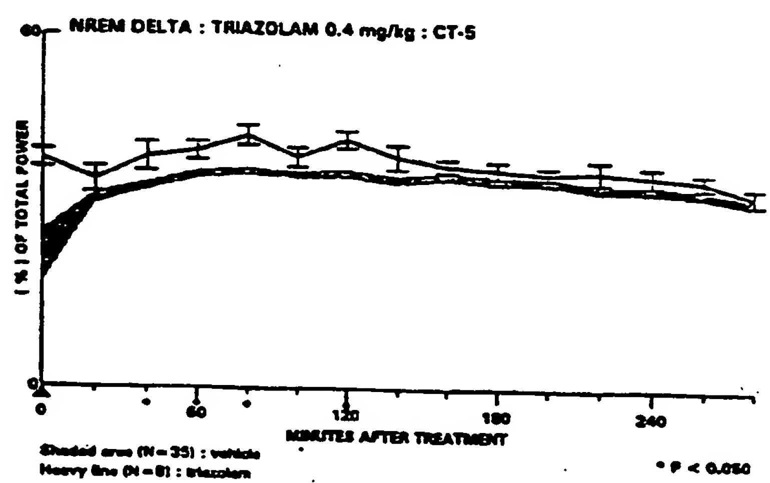


FIG. X C

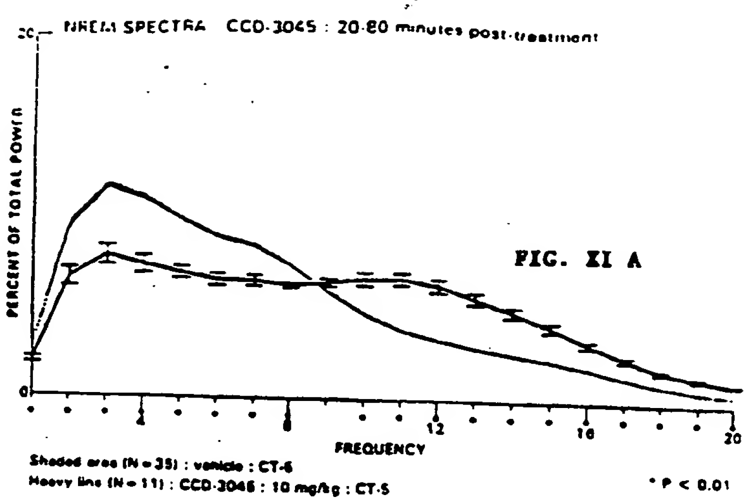


FIG. XI A

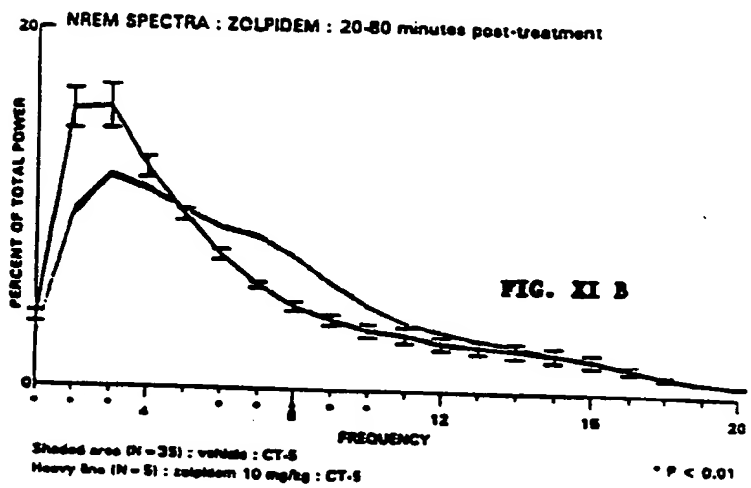


FIG. XI B

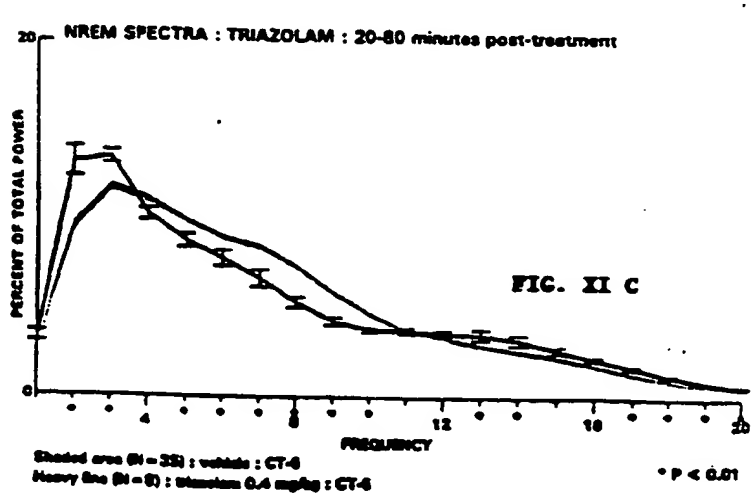


FIG. XI C

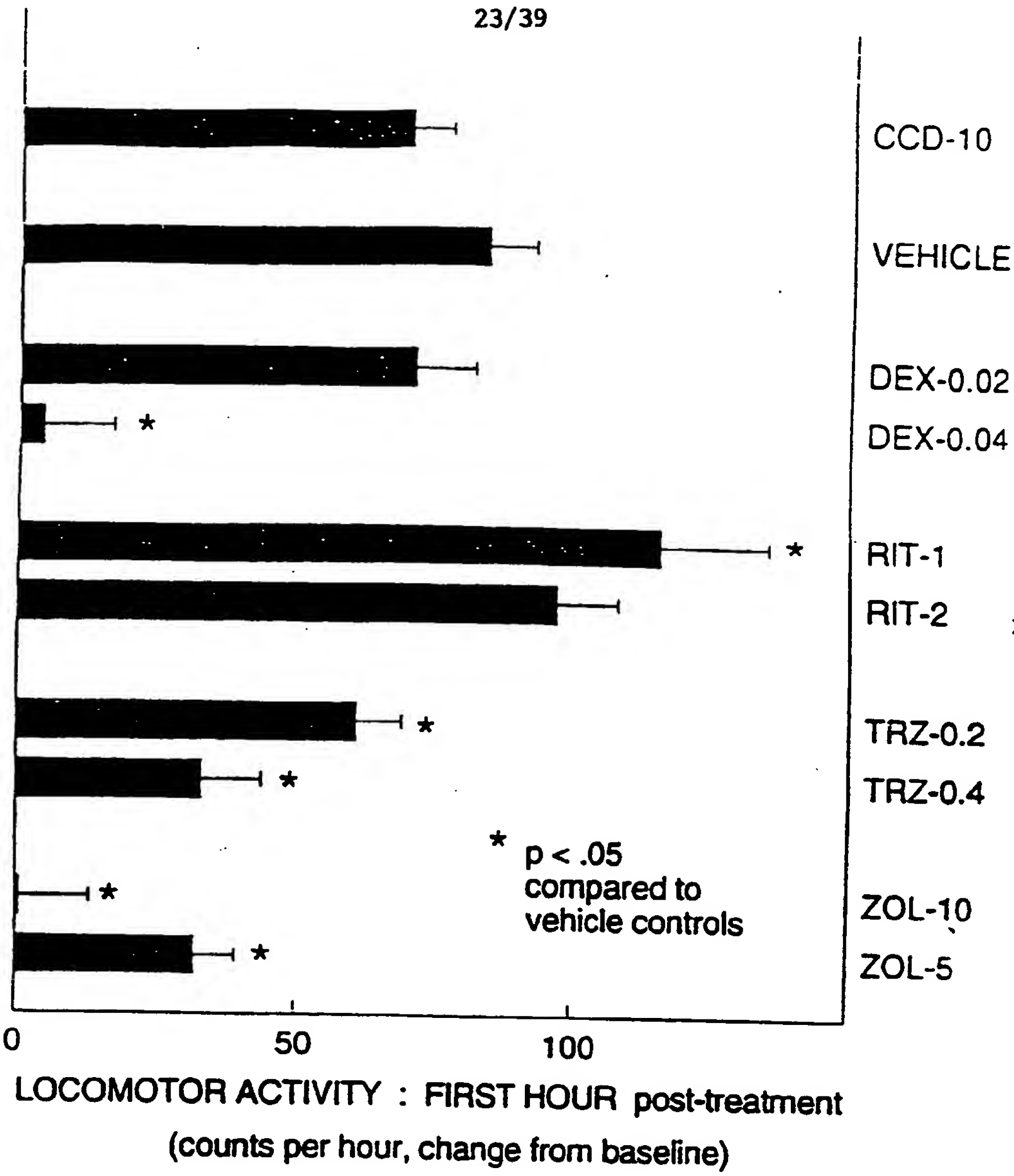

CCD-10
VEHICLE
DEX-0.02
DEX-0.04
RIT-1
RIT-2
TRZ-0.2
TRZ-0.4
ZOL-10
ZOL-5
* p < .05
compared to
vehicle controls
0
50
100
LOCOMOTOR ACTIVITY : FIRST HOUR post-treatment
(counts per hour, change from baseline)

FIG. XII

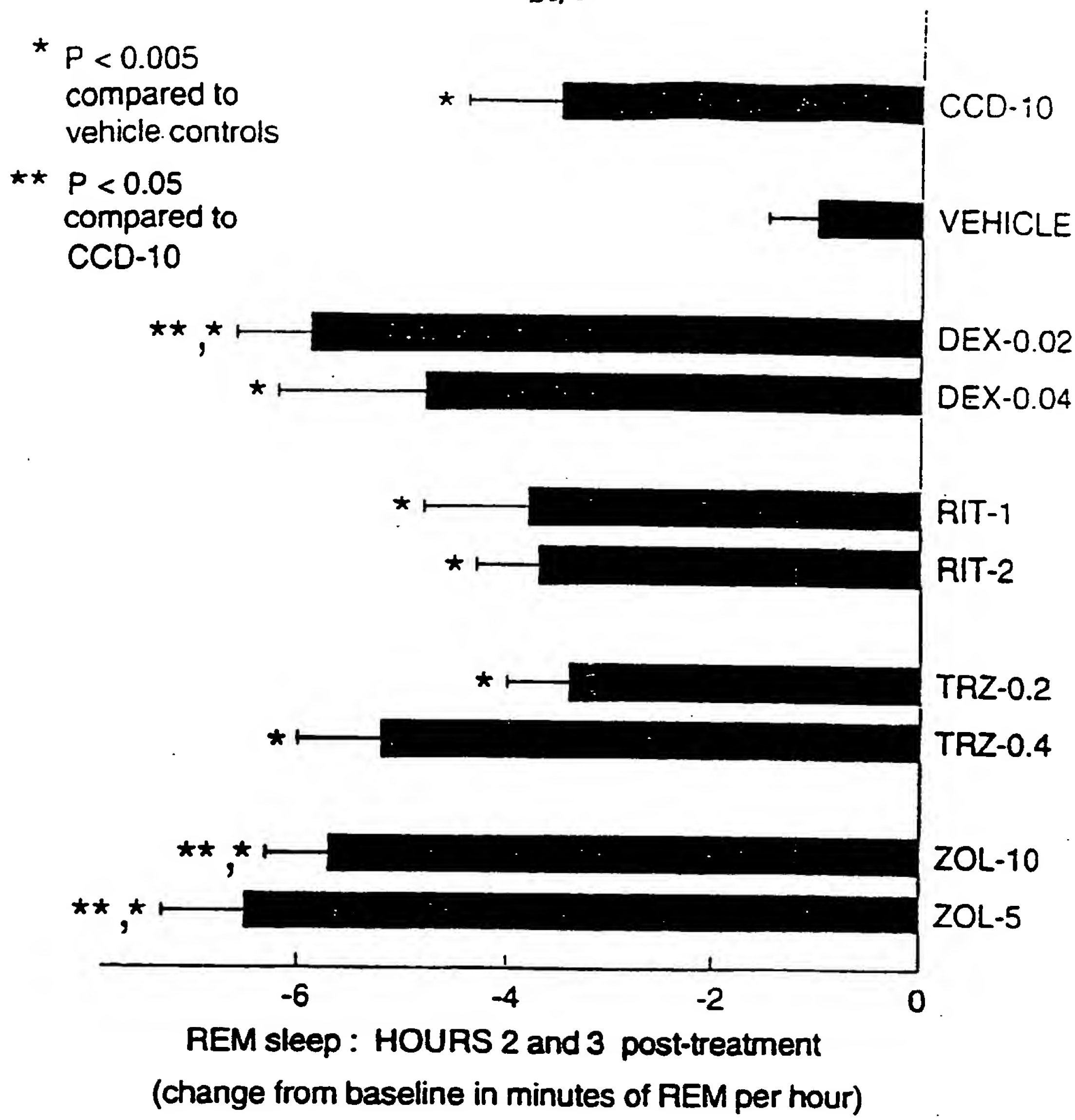

* P < 0.005
compared to
vehicle controls
** P < 0.05
compared to
CCD-10
CCD-10
VEHICLE
DEX-0.02
DEX-0.04
RIT-1
RIT-2
TRZ-0.2
TRZ-0.4
ZOL-10
ZOL-5
-6
-4
-2
0
REM sleep : HOURS 2 and 3 post-treatment
(change from baseline in minutes of REM per hour)

FIG. XIII

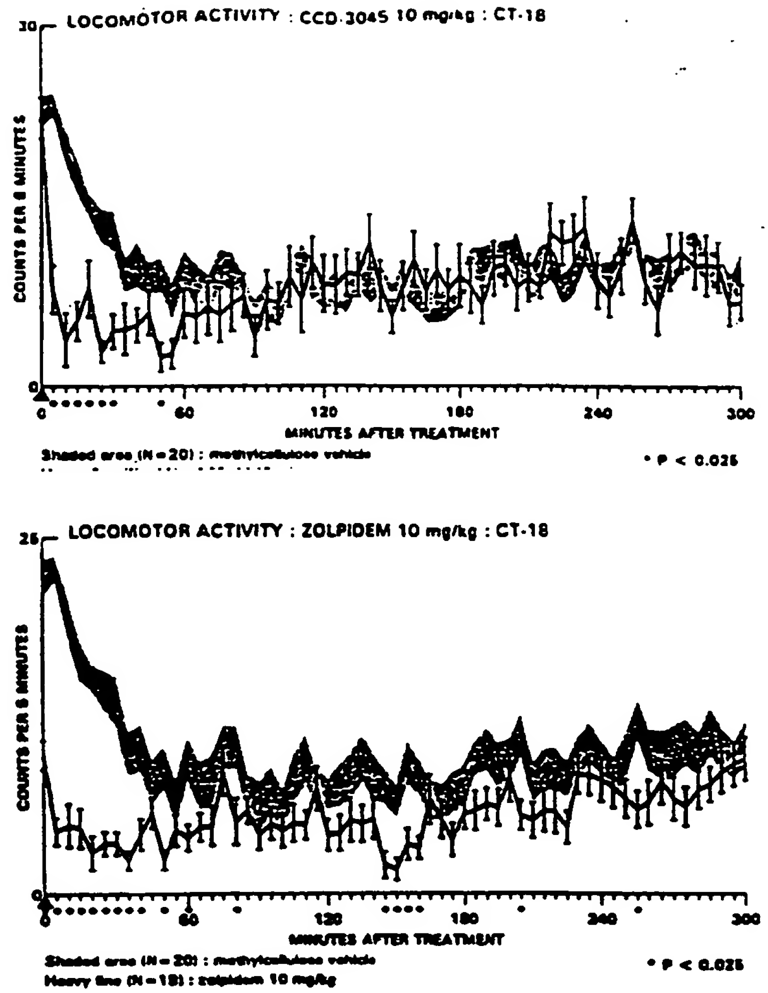
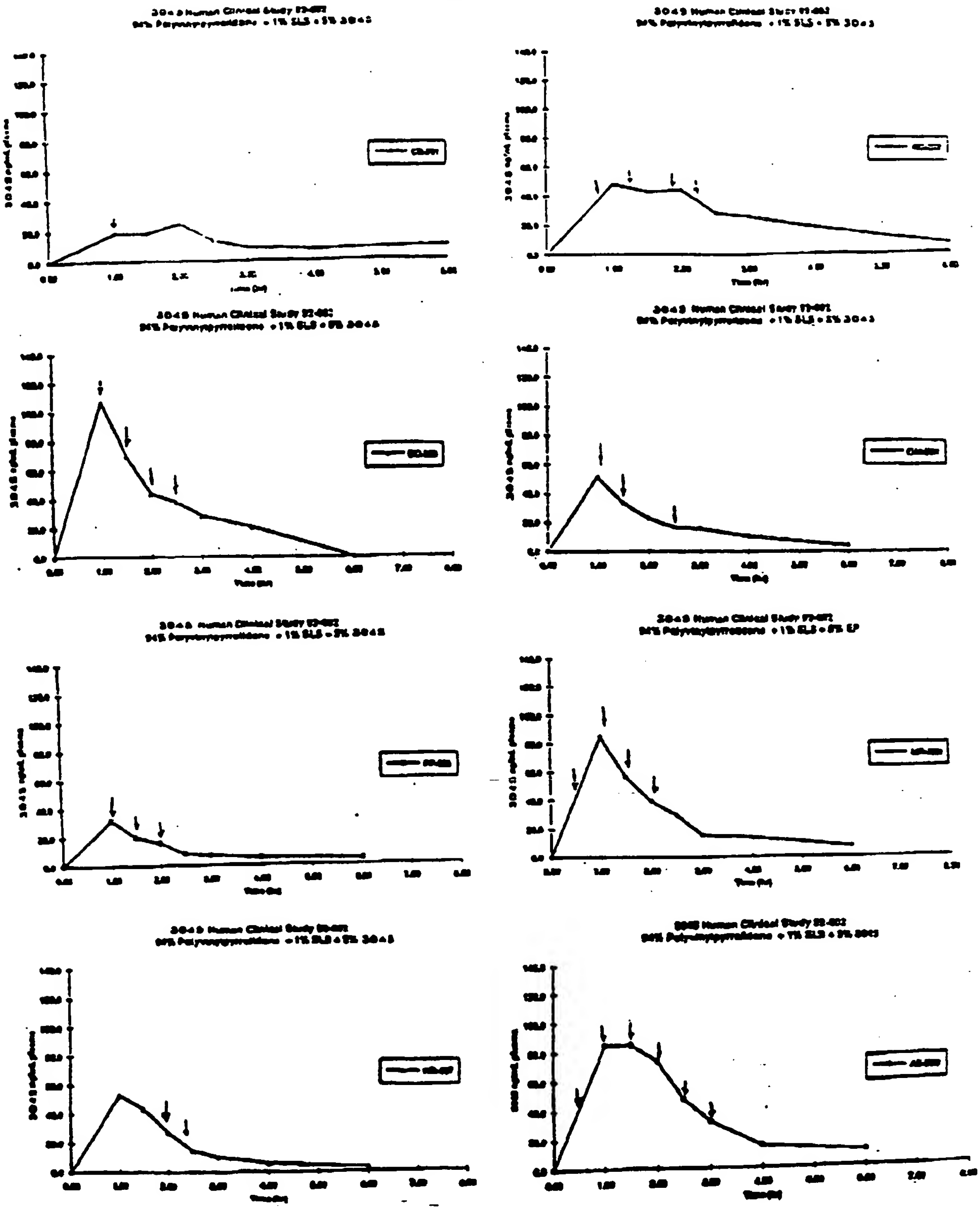

LOCOMOTOR ACTIVITY : CCD-3045 10 mg/kg : CT-18
30
0
COUNTS PER 6 MINUTES
0
60
120
180
240
300
MINUTES AFTER TREATMENT
Shaded area (N=20) : methylcellulose vehicle
• P < 0.025
LOCOMOTOR ACTIVITY : ZOLPIDEM 10 mg/kg : CT-18
25
0
COUNTS PER 6 MINUTES
0
60
120
180
240
300
MINUTES AFTER TREATMENT
Shaded area (N=20) : methylcellulose vehicle
Heavy line (N=18) : zolpidem 10 mg/kg
• P < 0.025

FIG. XIV

SUMMARY OF FORMULATION #1
94% POLYVINYLPYRROLIDONE + 1% SLS + 5% 3045

CCD 3045 HUMAN CLINICAL STUDY 92-002

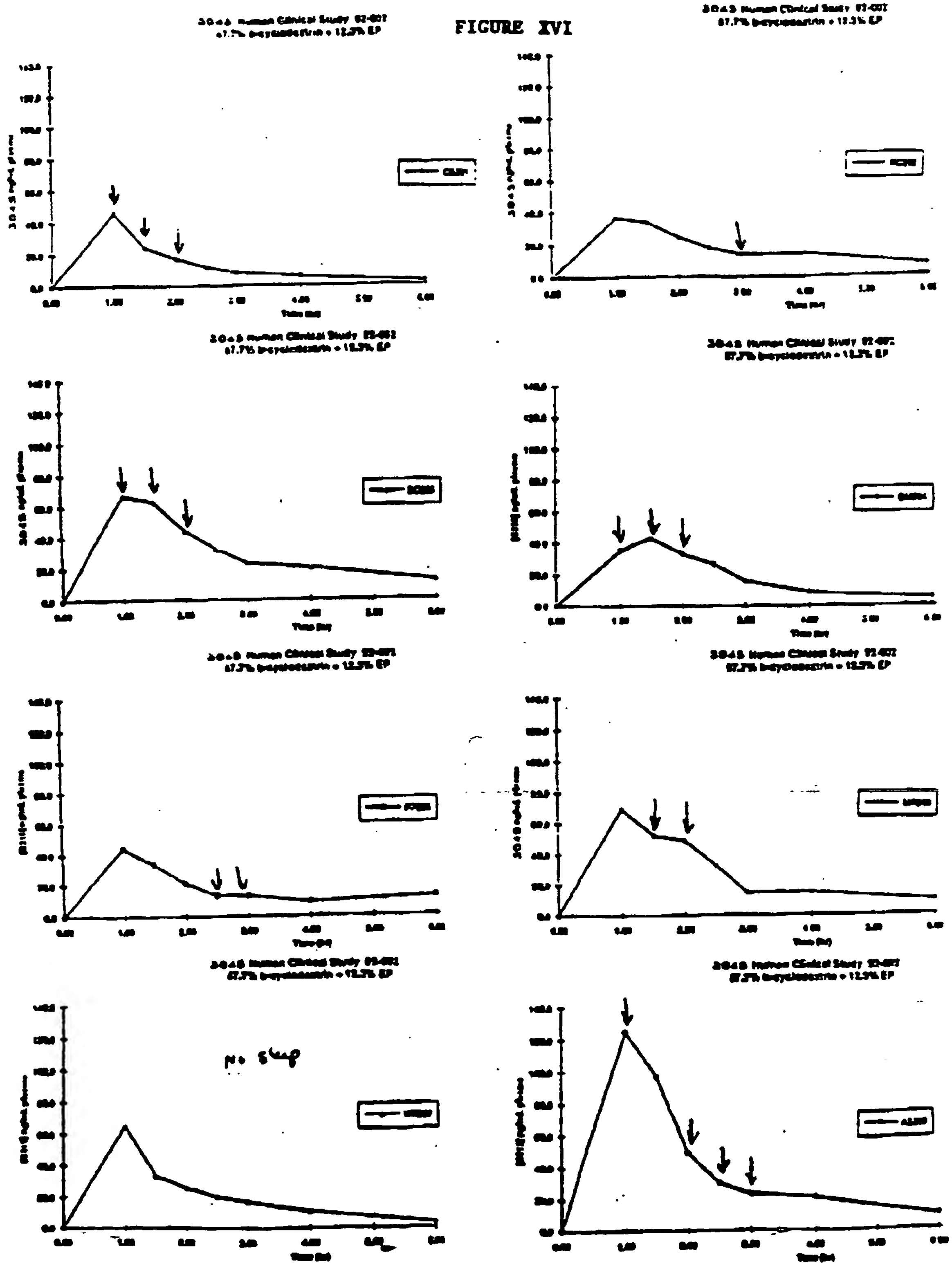
FIGURE XV



SUMMARY OF FORMULATION #2
INCLUSION COMPLEX: 12.3% 3045, 87.7% BETA-CYCLODEXTRIN

CCD 3045 HUMAN CLINICAL STUDY 92-002

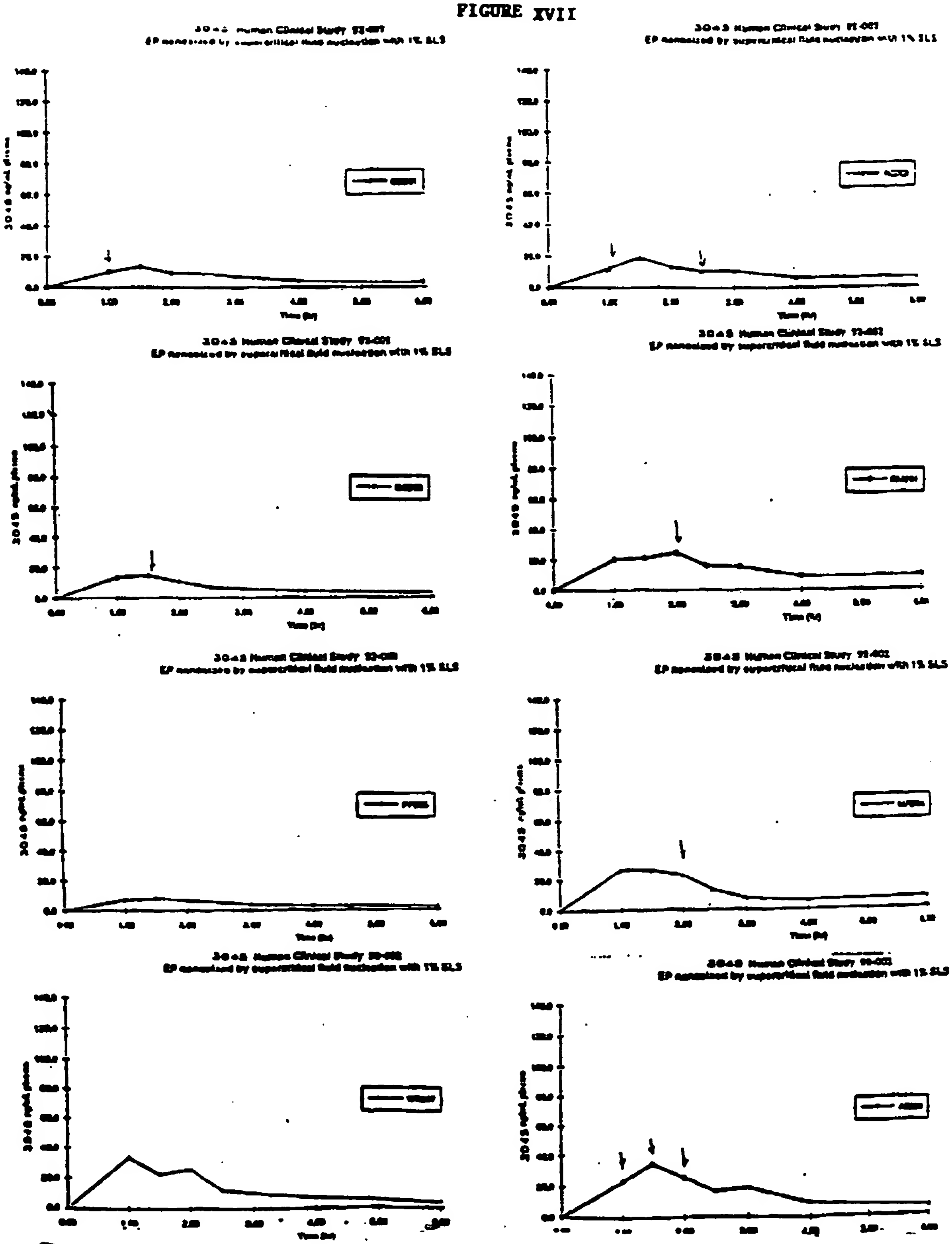
FIGURE XVI



SUMMARY OF FORMULATION #3
3045 NANOSIZED BY SUPERCRITICAL FLUID NUCLEATION WITH 1% SLS

CCD 3045 HUMAN CLINICAL STUDY 92-002

FIGURE XVII



SUMMARY OF FORMULATION #4
MICRONIZED 3045 (AKZO) WITH 1% SLS

CCD 3045 HUMAN CLINICAL STUDY 92-002

FIGURE XVIII

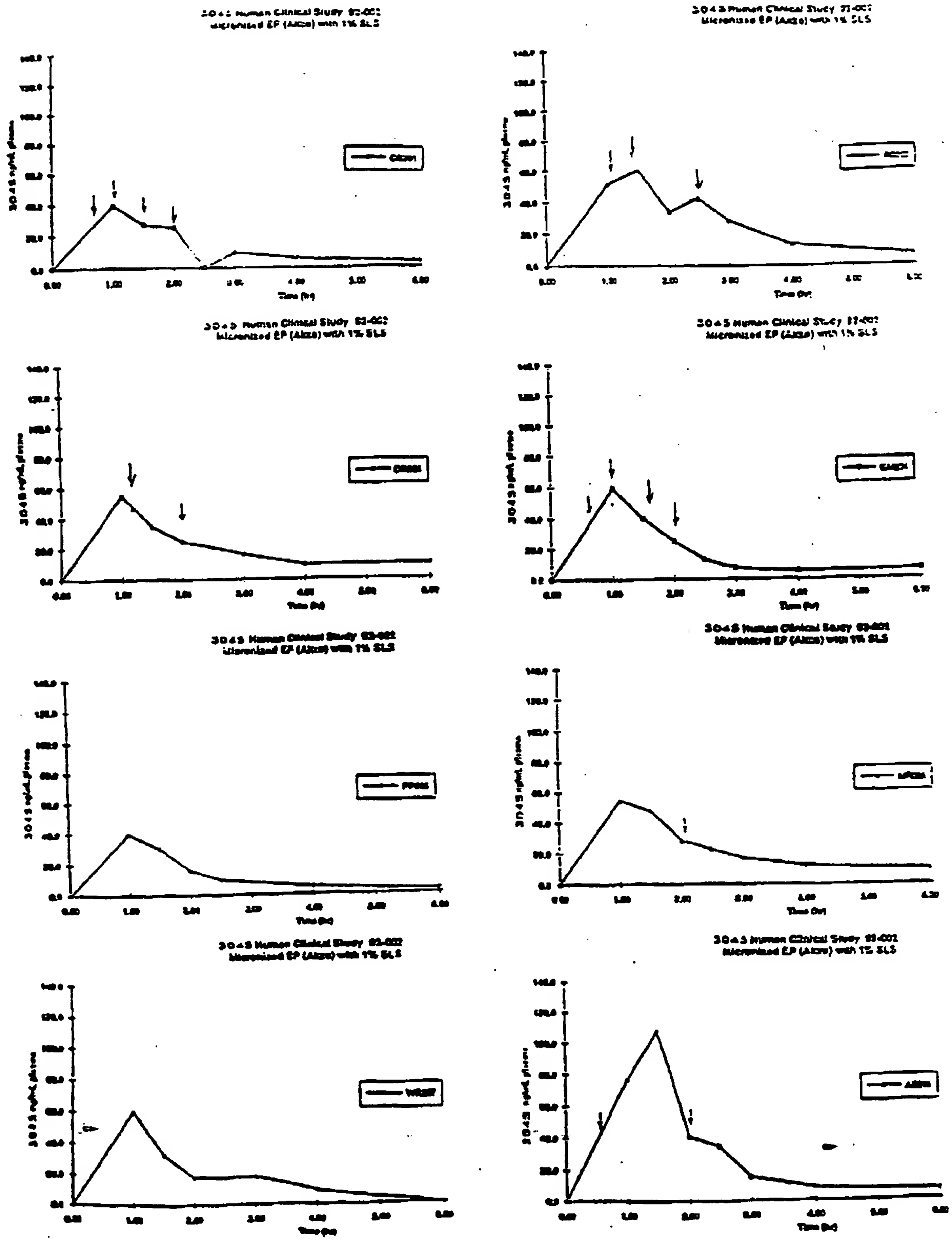


FIGURE XIX

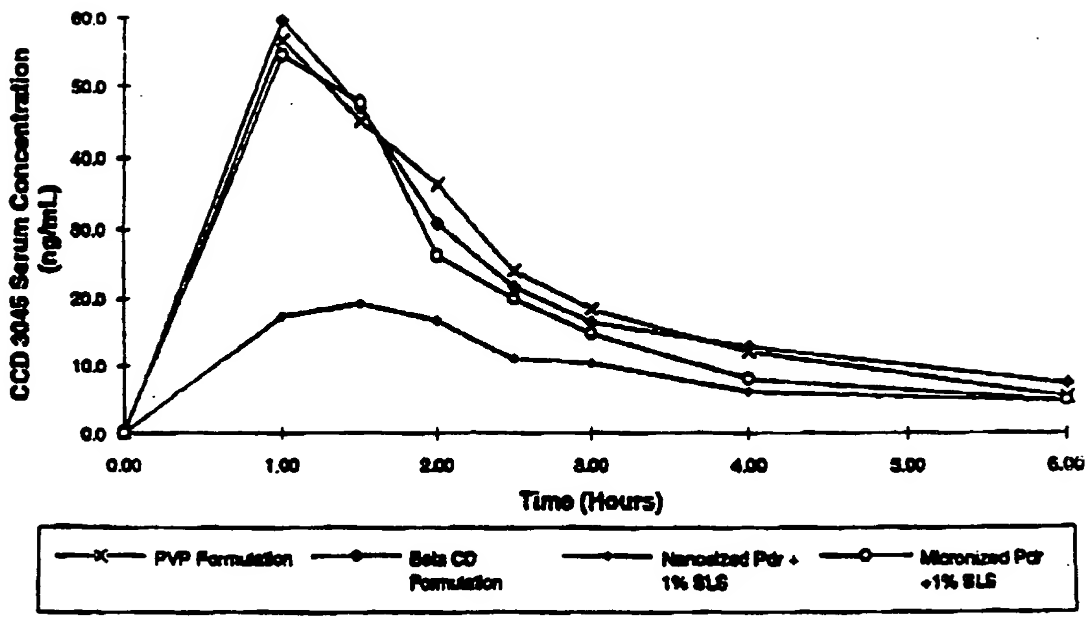

CCD 3045 Serum Concentration (ng/mL)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Time (Hours)
PVP Formulation
Beta CD Formulation
Nanosized Pdr + 1% SLS
Micronized Pdr +1% SLS

FIG. XX

Plasma concentration (ng/ml). Descriptive statistics

	Time (h) relative to intake	Mean	SD	n	Minimum	Median	Maximum
Formulation A	0	1.22	0.66	18	0.0	1.31	2.2
	0.5	48.92	42.20	18	8.9	35.21	176.6
	1	82.09	57.18	18	20.5	77.79	239.5
	1.5	79.33	49.71	18	24.5	71.06	173.7
	2	74.21	37.21	18	28.6	66.81	148.2
Formulation B	0	1.29	0.71	18	0.0	1.18	2.8
	0.5	21.27	25.21	18	2.0	6.80	82.8
	1	47.58	47.37	18	5.5	18.59	170.0
	1.5	64.06	54.91	18	9.8	41.60	192.2
	2	66.63	49.60	18	13.8	48.05	164.9

Based on table 2 of appendix 12.5.

Formulation A: CCD-3045 inclusion complex utilizing B-cyclodextrin
Formulation B: CCD-3045 plus sodium lauryl sulfate

FIG. XXI

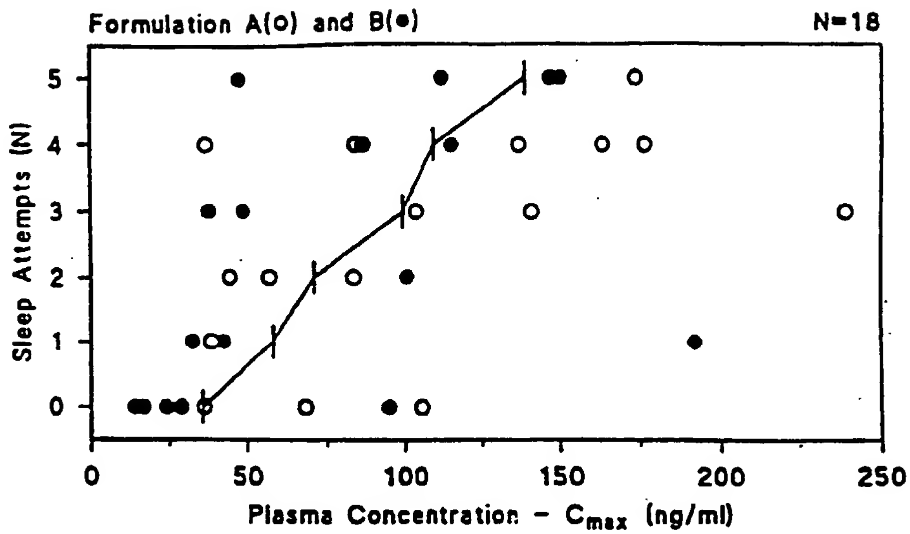
Time awake during the 5-min polygraphic recording

		Polygraphic recording: Wake						
Time Interval		Mean	SD	N	Trimean	1.Quart	Median	3.Quart
0	~~A-R1~~	4.87	0.35	18	5.00	5.00	5.00	5.00
30	~~A-R2~~	3.89	1.43	18	4.33	3.33	4.50	5.00
60	~~A-R3~~	2.92	1.95	18	3.04	1.33	2.92	5.00
90	~~A-R4~~	3.68	1.71	18	4.17	2.00	4.83	5.00
120	~~A-R5~~	3.98	1.51	18	4.54	3.50	4.84	5.00
0	~~B-R1~~	4.48	1.21	18	4.96	4.83	5.00	5.00
30	~~B-R2~~	4.02	1.48	18	4.50	3.00	5.00	5.00
60	~~B-R3~~	3.28	2.01	18	3.75	1.00	4.50	5.00
90	~~B-R4~~	3.63	1.46	18	3.84	2.50	3.92	5.00
120	~~B-R5~~	3.43	1.90	18	4.04	1.17	5.00	5.00

A and B in the left-side column of the table indicate the two formulations (A = Formulation I; B = Formulation II).

R1 to R5 represent the measurement times (R1 = time 0, R2 = 30 min post, R3 = 60 min post, R4 = 90 min post and R5 = 120 min post dosing.

FIG. XXII A



Relationship between C_{max} and sleep attempts within the 5-min recording sessions. Open circles represent formulation A, closed circles formulation B. Additionally, trimeans are represented by vertical bars.

FIG. XXII B

Number of sleep attempts	Sample size	Plasma Conc. (Trimean)
0	N = 10	35.5
1	N = 4	58.2
2	N = 4	71.0
3	N = 5	99.3
4	N = 8	109.6
5	N = 5	139.1

FIG. XXIII A

EEG NREM Recordings in Rat (min)				
Compound	Route	Dose(mg/kg)	Mean	SEM
3a-Hydroxy-3b-trifluoromethyl-5b-pregnan-20-one 21-phosphate disodium salt	PO	60	9.2	1.69
3b-ethynyl-3a-hydroxy-5b-pregnan-20-one	PO	18	26.7	4.05
3a-hydroxy-3b-methyl-5a-pregnan-20-one	IP	15	21.7	1.61
3a,21-Dihydroxy-3b-trifluoromethyl-5b-pregnan-20-one, 21-hemisuccinate, sodium sa	PO	60	13.2	1.64
3a-hydroxy-3b-ethynyl-5a-pregnan-20-one	PO	10	8.4	2.12
3a-hydroxy-3b-ethynyl-5a-pregnan-20-one	PO	25	34.7	2.19
3a,21-Dihydroxy-3b-ethynyl-5b-pregnan-20-one 21 hemisuccinate	PO	30	11.6	1.67
3a-Hydroxy-3b-trifluoromethyl-19-nor-5b-pregnan-20-one	PO	30	16.1	2.28
3a-Hydroxy-21-(pyrid-4-ylthio)-5b-pregnan-20-one	IP	30	33.2	3.5
3a-Hydroxy-3b-trifluoromethyl-5b-19-nor-pregnan-20-one 21-phosphate disodium sal	PO	30	9.3	1.4
10% Hydroxypropyl-b-cyclodextrin	PO	n/a	1.7	0.71
Water	PO	n/a	2.1	0.42

Definitions:

NREM (minutes): hourly-mean change-from-baseline during the first 5 hours post-treatment in NREM sleep.

FIG. XXIII B

EEG Rebound Recordings in Rat (min)				
Compound	Route	Dose(mg/kg)	Mean	SEM
3a-Hydroxy-3b-trifluoromethyl-5b-pregnan-20-one 21-phosphate disodium salt	PO	60	-0.25	0.39
3b-ethynyl-3a-hydroxy-5b-pregnan-20-one	PO	18	-0.34	0.60
3a-hydroxy-3b-methyl-5a-pregnan-20-one	IP	15	0.76	0.24
3a,21-Dihydroxy-3b-trifluoromethyl-5b-pregnan-20-one, 21-hemisuccinate, sodium salt	PO	60	-0.02	0.37
3a-hydroxy-3b-ethynyl-5a-pregnan-20-one	PO	10	-0.10	0.50
3a-hydroxy-3b-ethynyl-5a-pregnan-20-one	PO	25	0.74	0.64
3a,21-Dihydroxy-3b-ethynyl-5b-pregnan-20-one 21 hemisuccinate	PO	30	0.22	0.74
3a-Hydroxy-3b-trifluoromethyl-19-nor-5b-pregnan-20-one	PO	30	0.07	0.46
3a-Hydroxy-21-(pyrid-4-ylthio)-5b-pregnan-20-one	IP	30	1.45	0.60
3a-Hydroxy-3b-trifluoromethyl-5b-19-nor-pregnan-20-one 21-phosphate disodium salt	PO	30	-0.39	0.40
10% Hydroxypropyl-b-cyclodextrin	PO	n/a	1.10	0.60
Water	PO	n/a	0.13	0.18

REBOUND (minutes): hourly-mean change-from-baseline during the "rebound interval."

Baseline: the corresponding time period 24 hours earlier.

Rebound interval: the period that began at the cessation of the active compounds NREM-promoting effect, and continuing until 30 hours post-treatment. Cessation of activity was determined by inspection of the graph of hourly-averaged NREM sleep.

FIG. XXIII C

EEG REM Recordings in Rat (min)				
Compound	Route	Dose(mg/kg)	Mean	SEM
3a-Hydroxy-3b-trifluoromethyl-5b-pregnan-20-one 21-phosphate disodium salt	PO	60	-0.35	0.68
3b-ethynyl-3a-hydroxy-5b-pregnan-20-one	PO	18	-0.82	0.43
3a-hydroxy-3b-methyl-5a-pregnan-20-one	IP	15	-0.16	0.39
3a,21-Dihydroxy-3b-trifluoromethyl-5b-pregnan-20-one, 21-hemisuccinate, sodium	PO	60	-1.02	0.42
3a-hydroxy-3b-ethynyl-5a-pregnan-20-one	PO	10	0.63	0.32
3a-hydroxy-3b-ethynyl-5a-pregnan-20-one	PO	25	-0.67	0.34
3a,21-Dihydroxy-3b-ethynyl-5b-pregnan-20-one 21 hemisuccinate	PO	30	-1.17	0.46
3a-Hydroxy-3b-trifluoromethyl-19-nor-5b-pregnan-20-one	PO	30	-0.61	0.26
3a-Hydroxy-21-(pyrid-4-ylthio)-5b-pregnan-20-one	IP	30	-0.65	0.36
3a-Hydroxy-3b-trifluoromethyl-5b-19-nor-pregnan-20-one 21-phosphate disodium s	PO	30	-0.75	0.36
10% Hydroxypropyl-b-cyclodextrin	PO	n/a	-0.35	0.31
Water	PO	n/a	-0.19	0.21

REM (minutes): hourly-mean change-from-baseline during the first 5 hours post-treatment in REM sleep.

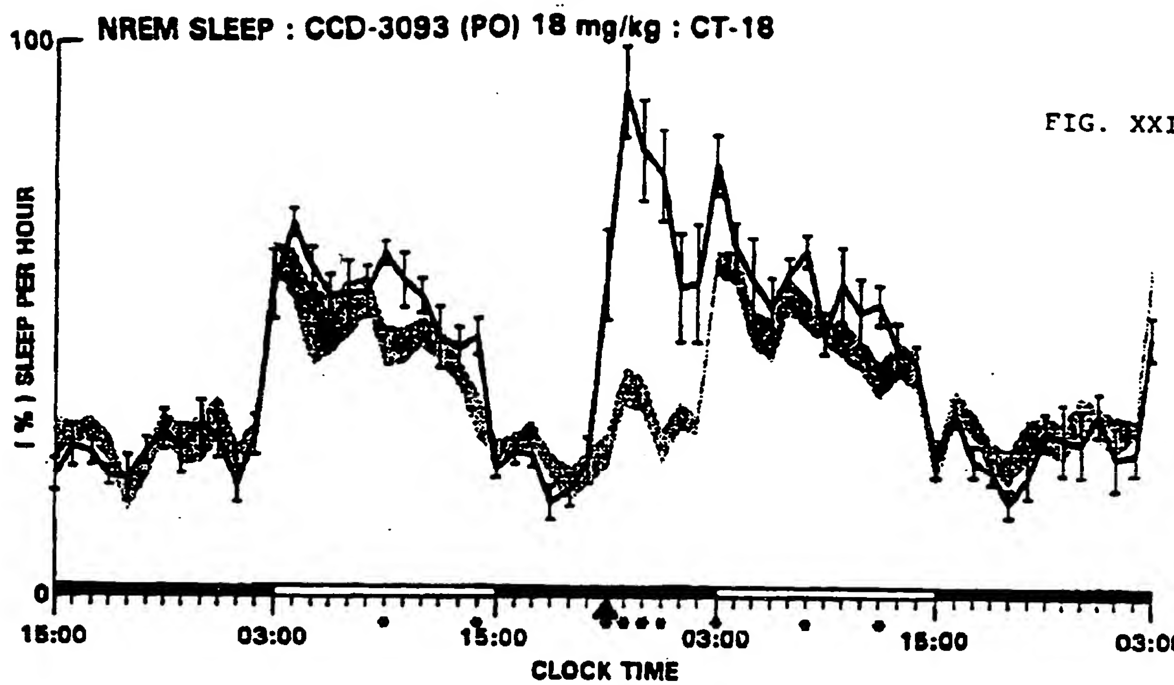


FIG. XXIV A

CCD-3093 is 3β-ethyl-3α-hydroxy-5β-pregnan-20-one

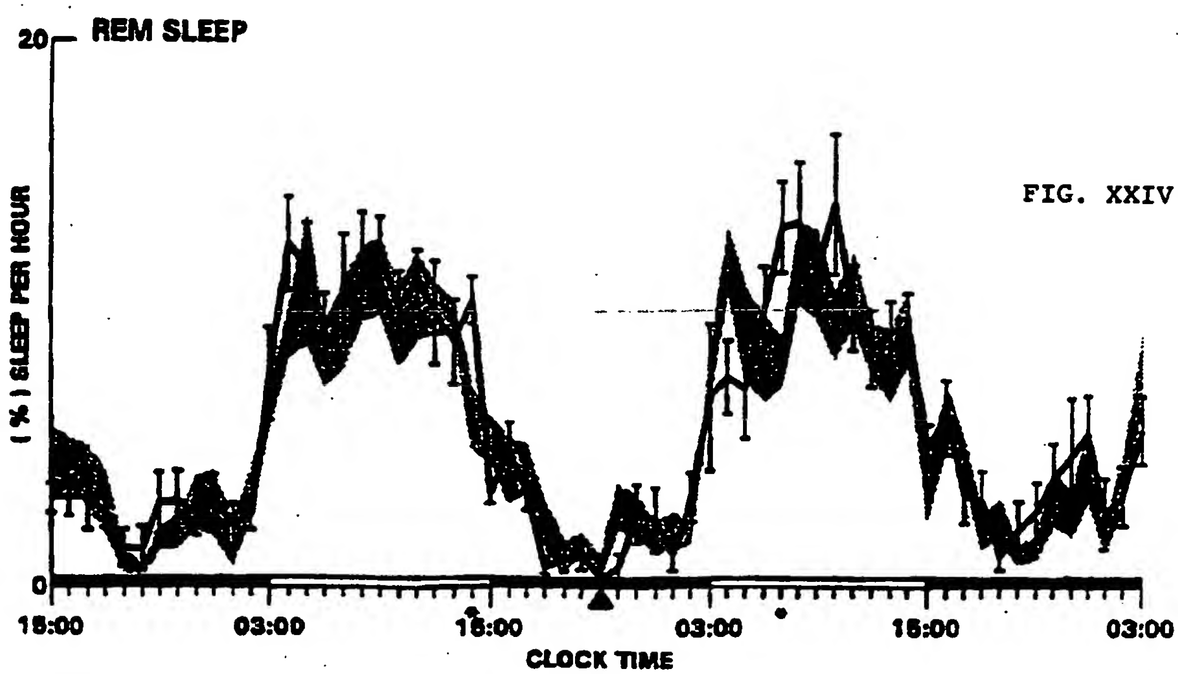


FIG. XXIV B

Shaded area (N = 10) : 10%HPBCD (PO) 10 ml/kg
Heavy line (N = 7) : CCD-3093 (PO) 18 mg/kg

* P < 0.025

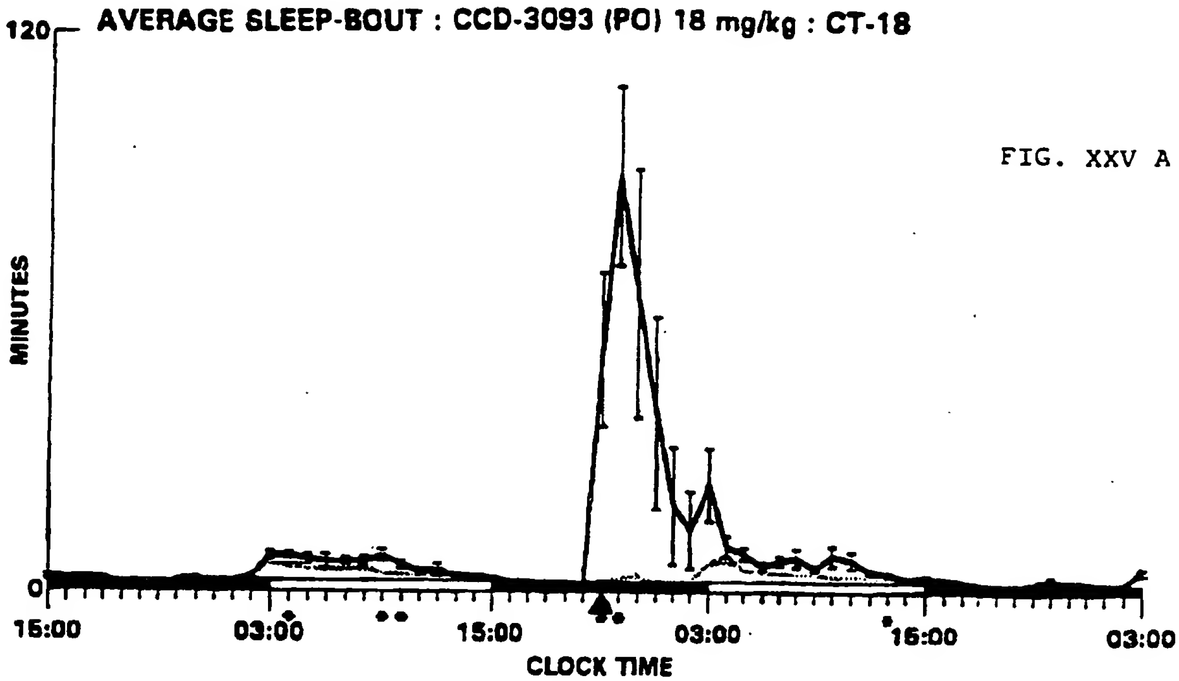

AVERAGE SLEEP-BOUT : CCD-3093 (PO) 18 mg/kg : CT-18
120
0
MINUTES
15:00
03:00
15:00
03:00
15:00
03:00
CLOCK TIME

FIG. XXV A

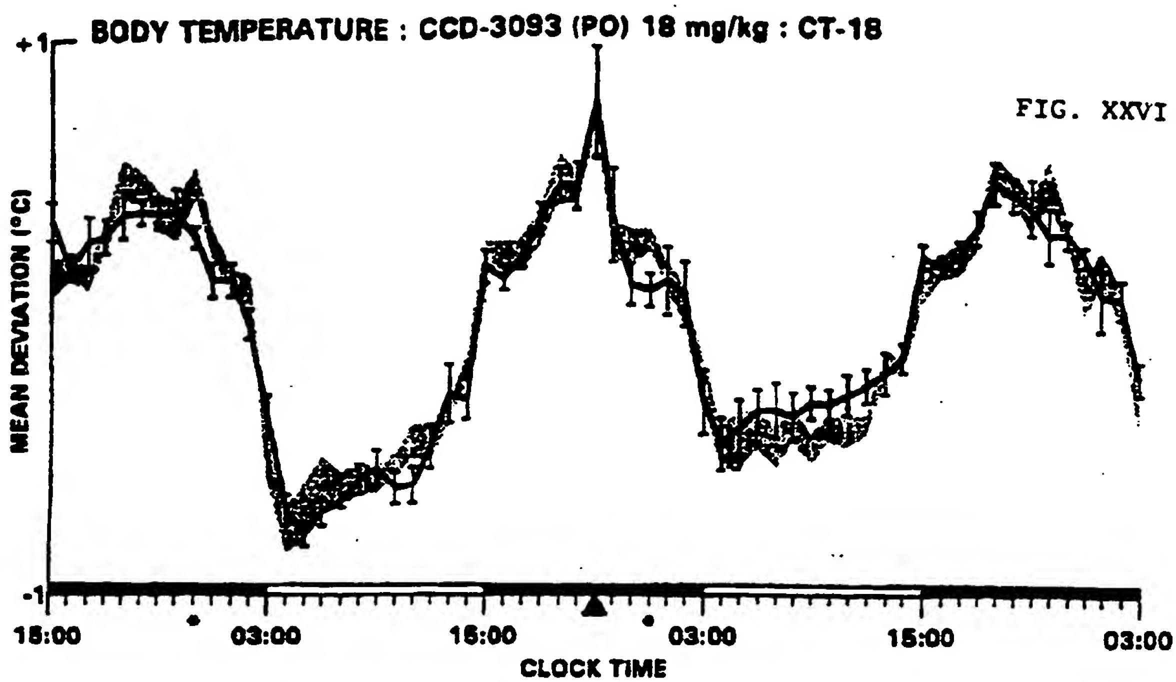


FIG. XXVI A

Shaded area (N=10) : 10%HPBCD (PO) 10 ml/kg
Heavy line (N=6) : CCD-3093 (PO) 18 mg/kg

* P < 0.025

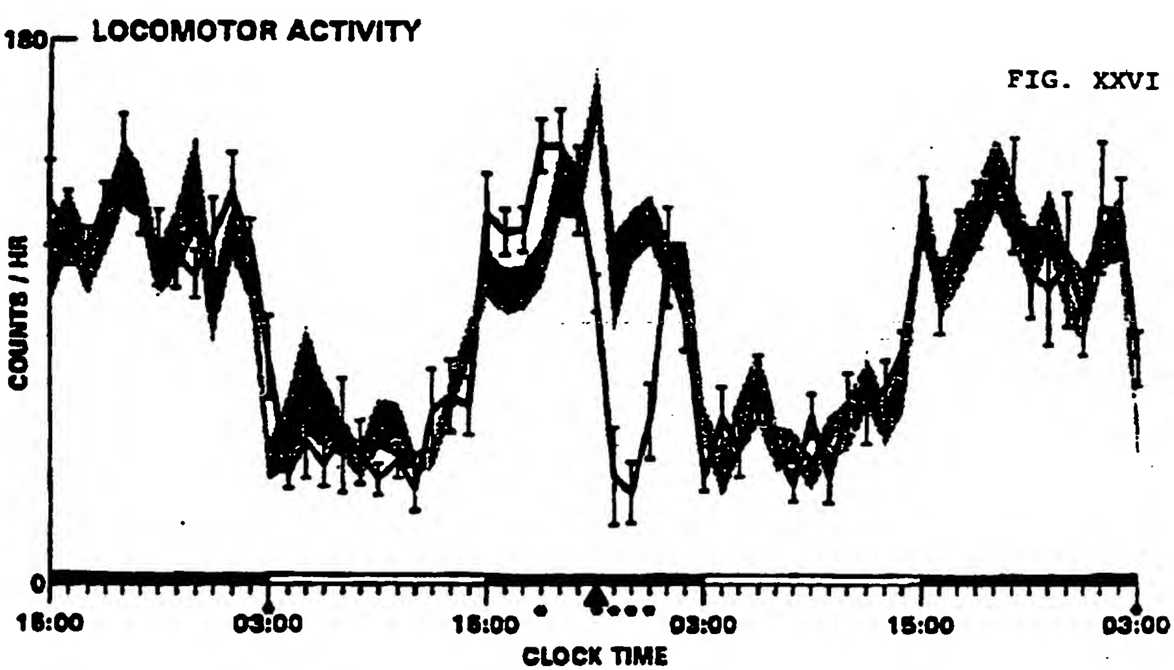


FIG. XXVI B

Shaded area (N=11) : 10%HPBCD (PO) 10 ml/kg
Heavy line (N=7) : CCD-3093 (PO) 18 mg/kg

* P < 0.025

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/05820

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/56 A61K31/57 A61K31/575 A61K31/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 05786 (COCENSYS, INC.) 1 April 1993 see page 26, line 7 - line 19 see claim 10 ---	1-26
X	WO,A,93 03732 (COCENSYS, INC.) 4 March 1993 see the whole document ---	1-26
X	WO,A,91 16897 (K.W.GEE ET AL.) 14 November 1991 see the whole document ---	1-26
X	WO,A,89 02272 (K.W.GEE) 23 March 1989 see the whole document --- -/--	1-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 1994	02.09.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Theuns, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEM.PHARMACOL., vol.21, no.5, 1972 pages 657 - 671 L.F.SOYKA ET AL. 'DISTRIBUTION AND HEPATIC METABOLISM OF PREGNANOLONE IN THE RAT' see page 667; table 6 ---	1-26
X	BRAIN RES. BULL., vol.29, no.1, 1992 pages 57 - 68 B.J.BOWERS ET AL. 'Biochemical and Behavioral Effects of Steroids on GABA-A Receptor Function in Long- and Short-Sleep Mice' see the whole document ---	1-26
X	PSYCHOPHARMACOLOGY, vol.93, no.2, 1987 pages 226 - 229 W.B.MENDELSON ET AL. 'Sleep induction by an adrenal steroid in the rat' see abstract ---	1-26
X	PHARMACOL.BIOCHEM.BEHAV., vol.42, no.4, August 1992 pages 605 - 611 C.L.MELCHIOR ET AL. 'Interaction of Pregnanolone and Pregnenolone Sulfate With Ethanol and Pentobarbital' see page 608 - page 609; figure 7 ---	1-26
X	PROC.SOC.EXP.BIOL.MED., vol.125, 1967 pages 1058 - 1062 L. GYERMEK 'Pregnanolone: A Highly Potent, Naturally Occurring Hypnotic-Anesthetic Agent.' cited in the application see the whole document ---	1-26
X	AM.J.OBSTET.GYNECOL., vol.159, November 1988 pages 1203 - 1209 E.S.ARAFAT ET AL. 'Sedative and hypnotic effects of oral administration of micronized progesterone may be mediated through its metabolites' cited in the application see the whole document ---	1-26

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

	Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
1	X	ANESTHESIOLOGY, vol.28, 1967 pages 173 - 183 G.HEUSER 'Induction of Anesthesia, Seizures and Sleep by Steroid Hormones' cited in the application see the whole document ---	1-26
1	X	SCIENCE, vol.232, no.4753, 23 May 1986 pages 1004 - 1007 M.D.MAJEWSKA ET AL. 'Steroid Hormone Metabolites Are Barbiturate-Like Modulators of the GABA Receptor' cited in the application see the whole document -----	1-26

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International application No.
PCT/US94/05820

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-26 are directed to a method of treatment of the human/animal body the search has been based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US 94/05820

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9305786	01-04-93	AU-A- 2657292	27-04-93
		CA-A- 2118938	01-04-93
		EP-A- 0603312	29-06-94
WO-A-9303732	04-03-93	US-A- 5232917	03-08-93
		AU-A- 2471992	16-03-93
		CA-A- 2093808	14-02-93
		EP-A- 0554436	11-08-93
		JP-T- 6501962	03-03-94
		US-A- 5319115	07-06-94
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		AU-A- 7771491	27-11-91
		EP-A- 0527962	24-02-93
		US-A- 5232917	03-08-93
		US-A- 5319115	07-06-94
WO-A-8902272	23-03-89	AU-B- 609927	09-05-91
		AU-A- 2828389	17-04-89
		EP-A- 0382790	22-08-90
		JP-T- 3500049	10-01-91
		US-A- 5120723	09-06-92
		US-A- 5208227	04-05-93
		US-A- 5232917	03-08-93
		US-A- 5319115	07-06-94